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Term: L15 and acryloyl

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Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT; PLUR=YES; OP=ADJ		
L16	L15 and acryloyl	15	L16
L15	L14 and pores and electrode	86	L15
L14	surfactant and acrylamide and hydrogel	1029	L14
L13	porous hydrogen and porogen and monomer	0	L13
L12	mesoporous hydrogel and acrylamide	0	L12
L11	l1 and surfactant and mesoporous	0	L11
L10	L8 and pores	4	L10
L9	L8and pores	0	L9
L8	l1 and surfactant	13	L8
L7	L1 and porogen	0	L7
L6	L3 and porogen	0	L6
L5	L4 and porogen	0	L5
L4	L3 and pores	3	L4
L3	L2 and silica	6	L3
L2	L1 and acrylamide	29	L2
L1	hydrogel layer same electrode	92	L1

10/014,845

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Term: L6 and pores

Display: 10 **Documents in Display Format:** CIT **Starting with Number** 1

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L9	L6 and pores	24	L9
L8	L6and mesoporous	0	L8
L7	L6 and mesopores	0	L7
L6	L5 and substrate	78	L6
L5	L4 and polymer hydrogel	169	L5
L4	hydrogel and electrode and polymer	1457	L4
L3	L1 and electrode	2	L3
L2	L1 and layer and electrode	0	L2
L1	hydrogel same polymer and mesoporous	4	L1

END OF SEARCH HISTORY

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L3: Entry 1 of 2 File: USPT Sep 9, 2003

US-PAT-NO: 6615855
DOCUMENT-IDENTIFIER: US 6615855 B2

TITLE: Stimuli-responsive hybrid materials containing molecular actuators and their applications

DATE-ISSUED: September 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lopez; Gabriel	Albuquerque	NM		
Chilkoti; Ashutosh	Durham	NC		
Atanassov; Plamen	Albuquerque	NM		
Goparaju; Venkata	Albuquerque	NM		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Science & Technology Corporation @t UNM	Albuquerque	NM			02

APPL-NO: 10/ 238852 [PALM]
DATE FILED: September 11, 2002

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application makes reference to U.S. patent application Ser. No. 09/790,974, now U.S. Pat. No. 6,491,061, entitled "Stimuli-Responsive Hybrid Materials Containing Molecular Actuators and their Applications," filed Feb. 23, 2001, which claims priority to U.S. provisional Application No. 60/185,057, entitled "Stimuli-Responsive Hybrid Materials Containing Molecular Actuators and their Applications," filed Feb. 25, 2000. The entire contents and disclosures of these applications are hereby incorporated by reference.

INT-CL: [07] F16 K 11/02

US-CL-ISSUED: 137/2; 137/599.01, 137/468, 251/11
US-CL-CURRENT: 137/2; 137/468, 137/599.01, 251/11

FIELD-OF-SEARCH: 137/599.01, 137/601.13, 137/455, 137/468, 137/2

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected Search ALL Clear

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
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5416074	May 1995	Rabaud et al.	
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5643247	July 1997	Fernandez et al.	604/891.1
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Urry, Dan W., "Physical Chemistry of Biological Free Energy Transduction As Demonstrated by Elastic Protein-Based Polymers," J. Phys. Chem. B. 1997, 101, 11007-11028.

ART-UNIT: 3753

PRIMARY-EXAMINER: Hepperle; Stephen M.

ATTY-AGENT-FIRM: Jagtiani & Guttag

ABSTRACT:

The present invention provides a hybrid material and a method for forming a hybrid material comprising actuator made from a stimuli responsive polymer mounted in a porous framework. The present invention also provides devices employing the actuator of the present invention.

14 Claims, 5 Drawing figures

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Search Results - Record(s) 1 through 2 of 2 returned.

1. Document ID: US 6615855 B2

L3: Entry 1 of 2 File: USPT Sep 9, 2003

US-PAT-NO: 6615855
DOCUMENT-IDENTIFIER: US 6615855 B2

TITLE: Stimuli-responsive hybrid materials containing molecular actuators and their applications

DATE-ISSUED: September 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lopez; Gabriel	Albuquerque	NM		
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Atanassov; Plamen	Albuquerque	NM		
Goparaju; Venkata	Albuquerque	NM		

US-CL-CURRENT: 137/2; 137/468, 137/599.01, 251/11

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw D

2. Document ID: US 6491061 B1

L3: Entry 2 of 2 File: USPT Dec 10, 2002

US-PAT-NO: 6491061
DOCUMENT-IDENTIFIER: US 6491061 B1

TITLE: Stimuli responsive hybrid materials containing molecular actuators and their applications

DATE-ISSUED: December 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lopez; Gabriel	Albuquerque	NM		
Chilkoti; Ashutosh	Durham	NC		
Atanassov; Plamen	Albuquerque	NM		
Goparaju; Venkata	Albuquerque	NM		

US-CL-CURRENT: 137/599.01; 137/468, 251/11

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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Terms	Documents
L1 and electrode	2

Display Format: CIT Change Format

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L9: Entry 10 of 24 File: USPT Dec 5, 2000

DOCUMENT-IDENTIFIER: US 6156572 A
TITLE: Bioartificial extracellular matrix containing hydrogel matrix derivatized with cell adhesive peptide fragment

Abstract Text (1):
A bioartificial extracellular matrix for use in tissue regeneration or replacement is provided by derivatizing a three-dimensional hydrogel matrix with a cell adhesive extracellular matrix protein or cell adhesive peptide fragment of the protein. Preferably, derivatizing is by covalent immobilization of a cell adhesive peptide fragment having the amino acid sequence, ArgGlyAsp, TyrIleGlySerArg or IleLysValAlaVal. Cartilage or tendon can be regenerated by implanting a matrix containing an adhesive peptide fragment that favors chondrocyte invasion. The matrix can be pre-seeded with cells, and tissue can be reconstituted in vitro and then implanted. A cell-seeded matrix can be encapsulated in a semi-permeable membrane to form a bioartificial organ. An agarose hydrogel matrix having an agarose concentration of 0.5-1.25% (w/v) and an average pore radius between 120 nm and 290 nm is preferred. A nerve guidance channel for use in regenerating severed nerve is prepared containing a tubular semi-permeable membrane having openings adapted to receive ends of a severed nerve, and an inner lumen containing the hydrogel matrix having a bound cell adhesive peptide fragment through which the nerve can regenerate.

Brief Summary Text (13):
All of the studies using these preptidic sequences of cell attachment and neurite promotion were conducted on flat two-dimension substrates (Smallheiser et al., Dev. Brain Res., 12, pp. 136-40 (1984); Graf et al., Biochemistry, 26, pp. 6896-900 (1987); Sephel et al., Biochem. Biophys. Res. Comm., 2, pp. 821-29 (1989); Jucker et al., J. Neurosci. Res., 28, pp. 507-17 (1991)). The physical and chemical nature of the culture substrate influences cell attachment and neurite extension. The physical microstructure of a 2-D culture substrate can influence cell behavior. The use of permissive and on-permissive culture surface chemistries facilitates nerve guidance in 2-D. The cell attachment regulating function of various serum proteins like albumin and fibronectin is dependent on the chemistries of the culture substrates that they are adsorbed onto.

Brief Summary Text (14):
Gene expression is reported to be regulated differently by a flat 2-D substrate as opposed to a hydrated 3-D substrate. For example, monolayer culture of primary rabbit articular chondrocyte and human epiphyseal chondrocyte on 2-D tissue culture substrates causes primary chondrocyte to lose their differentiated phenotype. The differentiated chondrocyte phenotype is re-expressed when they are cultured in 3-D agarose gels (Benya and Shaffer, Cell, 30, pp. 215-24 (1982); Aulthouse, et al., In Vitro Cell Dev. Bio., 25, pp. 659-68 (1989)).

Brief Summary Text (19):
This invention provides a three-dimensional hydrogel based, biosynthetic, extracellular matrix (ECM) equivalent, and method of making same. Agarose matrices having a chemistry amenable to derivatization with various ECM adhesive peptides and proteins, are preferred in forming the 3-D hydrogel substrates of this invention. These biologically active 3-D templates may be useful in facilitating

tissue regeneration or replacement.

Drawing Description Text (2):
FIG. 1. A double Y-axis plot depicting the influence of agarose gel concentration on average pore radius (Y1) and percent striatal cells extending neurites (Y2) after 72 hours in culture. Pore radius was calculated by hydraulic permeability measurements of the different gel concentrations. Solid line through pore radii data points is an exponential fit with r.sup.2 =0.985.

Drawing Description Text (8):
FIG. 7. Graph showing the number of myelinated axons regenerated at 4 weeks along polymer guidance channels filled with AgPlain and Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) gels. "*" depicts a statistically significant difference with p<0.05 using the Student t test.

Drawing Description Text (9):
FIG. 8. Graph showing the density of myelinated axons regenerated at 4 weeks along polymer guidance channels filled with AgPlain and Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) gels. "*" depicts a statistically significant difference with p<0.05 using the Student t test.

Drawing Description Text (10):
FIG. 9. Histogram depicting the number of myelinated axons in regenerating sural nerves at 2.0 mm distance from the proximal nerve stump in polymer guidance channels filled with A) saline; B) AgPlain and C) Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1). "*" depicts a statistical difference of p<0.05 when compared to saline or AgPlain. Student t test was used to evaluate statistical significance.

Drawing Description Text (11):
FIG. 10. Histogram depicting the density of myelinated axons in regenerating sural nerves at 2.0 mm distance from the proximal nerve stump in polymer guidance channels filled with A) saline; B) AgPlain and C) Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1). "*" depicts a statistical difference of p<0.05 when compared to saline or AgPlain. Student t test was used to evaluate statistical significance.

Detailed Description Text (2):
This invention provides a biosynthetic, hydrogel-based, three-dimensional bioartificial ECM. The bioartificial extracellular matrices of this invention offer the possibility of manipulating cells in 3-D, and may be used as three dimensional templates for tissue engineering efforts in vitro and in vivo.

Detailed Description Text (5):
Any suitable hydrogel may be used as the substrate for the bioartificial extracellular matrices of this invention. Compositions that form hydrogels fall into three classes. The first class carries a net negative charge (e.g., alginate). The second class carries a net positive charge (e.g., collagen and laminin). Examples of commercially available extracellular matrix component hydrogels include Matrigel.TM. and Vitrogen.TM.. The third class is net neutral in charge (e.g., highly crosslinked polyethylene oxide, or polyvinylalcohol).

Detailed Description Text (6):
A hydrogel suitable for use in this invention is preferably a defined polymer, most preferably a polymer that is synthetic or can be prepared from a naturally occurring, non-tumorigenic source, free of undesired biological (e.g., bacterial or viral), chemical or other contaminants. Most preferred as the matrix substrate are well characterized hydrogels that permit presentation of only the desired ECM adhesion molecule or adhesive peptide fragement in 3-D, substantially free of undesired adhesion motifs.

Detailed Description Text (7):
Matrigel.TM. is not a defined substrate and also less desirable since it is derived from a murine sarcoma line. In addition, not all synthetic polymer hydrogels are suitable. For example, the use of acrylic based hydrogels by Woerly et al., Cell Transplantation, 2, pp. 229--39 (1993) presents the possibility of cytotoxicity because entrapment of neuronal cells is done concomitantly with the cross-linking reaction in the presence of free radical initiators.

Detailed Description Text (8):
Polymers that may be useful hydrogel matrix substrate materials include high molecular weight polyethylene oxide (PEO) and hyaluronate. Stabilized hyaluronate is commercially available (Fidia Advanced Biopolymers). Various PEO polymers are also commercially available.

Detailed Description Text (9):
Polysaccharides are a class of macromolecules of the general formula (CH.sub.2 O).sub.n which are useful as the hydrogel substrate in the present invention. Polysaccharides include several naturally occuring compounds, e.g., agarose, alginate and chitosan. We prefer agarose.

Detailed Description Text (10):
Agarose is a clear, thermoreversible hydrogel made of polysaccharides, mainly the alternating copolymers of 1,4 linked and 3,6-anhydro-.alpha.-L-galactose and 1,3 linked .beta.-D-galactose. Two commercially available agaroses are SeaPrep.RTM. and SeaPlaque.RTM. agarose (FMC Corp. Rockland, Me.). SeaPrep.RTM. is a hydroxyethylated agarose that gels at 17.degree. C. The particular suitability of a hydrogel as a biomaterial stems from the similarity of its physical properties to those of living tissues. This resemblance is based on its high water content, soft rubbery consistency and low interfacial tension. The thermoreversible properties of agarose gels make it possible for agarose to be a liquid at room temperature allowing for easy mixing of cell-gel solution and then cooling to 4.degree. C. causes gelation and entrapment of cells. This is a comparatively benign process, free of chemicals toxic to the cells.

Detailed Description Text (11):
We prefer an agarose concentration of 0.50 to 1.25% (w/v), most preferably 1.0%, for the permissive layers of the hydrogel matrix.

Detailed Description Text (12):
Several physical properties of the hydrogel matrices of this invention are dependent upon gel concentration. Increase in gel concentration may change the gel pore radius, morphology, or its permeability to different molecular weight proteins.

Detailed Description Text (13):
Gel pore radius determination can be determined by any suitable method, including hydraulic permeability determination using a graduated water column, transmission electron microscopy and sieving spheres of known radius through different agar gel concentrations. See, e.g., Griess et al., Biophysical J., 65, pp. 138-48 (1993). We prefer hydraulic permeability-based pore radius determination, as the method most sensitive to changes in gel concentration.

Detailed Description Text (14):
Measurement of gel hydraulic permeability using a graduated water column enabled the calculation of average pore radius for each of the gel concentrations studied. The average gel pore radius fall exponentially as the gel concentration increased. The slope of the curve indicated the sensitivity of pore radius to gel concentration. The average gel pore radius preferably varies between 120-290 nm, and is most preferably approximately 150 nm. The pore radius of the 1.25% threshold

agarose gel concentration was 150 nm.

Detailed Description Text (15):
The agarose hydrogels of this invention may be used as a carrier to present various ECM proteins or peptides, e.g., laminin fibronectin, and/or their peptidic analogs in 3-D. The chemistry of agarose permits easy modification with such ECM adhesive proteins and/or peptides. We prefer covalent immobilization of ECM proteins to the hydrogel backbone. Such immobilization is important because the physical blending of low molecular weight oligopeptides with hydrogels will not retain the peptides in the gel. Further, covalent immobilization prevents the possible saturation of cell surface receptors by `free-floating` ECM molecules in hydrogel-ECM molecule blends.

Detailed Description Text (17):
The bioartificial hydrogel extracellular matrices of this invention are useful for presenting in 3-D full length extracellular matrix proteins involved in cell adhesion. In addition, peptide fragments of such adhesion molecules that contain cell binding sequences may also be used (i.e., adhesive peptide fragement). Several such adhesive peptide fragments are known in the art. A particular peptide fragment can be tested for its binding ability or adhesive capacity according to standard techniques.

Detailed Description Text (18):
The bioartificial hydrogel matrices of this invention can be used to present ECM adhesion molecules, or adhesive peptide fragments thereof, in 3-D to a variety of cell types. These cell types include any cell that is normally in contact with the ECM in vivo, or any cell bearing a cell surface receptor capable of binding to an ECM adhesion molecule or adhesive peptide fragment thereof.

Detailed Description Text (24):
In some embodiments, the hydrogel ECM matrix can be derivatized with the appropriate ECM adhesion molecules or adhesive peptide fragments and implanted into a desired location in a host, e.g., a mammal, preferably a human. In these embodiments, the matrix acts as a support for tissue regeneration, whereby the host cells infiltrate the matrix. In the presence of the appropriate 3-D molecular cues in the matrix host tissue regeneration is facilitated.

Detailed Description Text (26):
In other embodiments, the bioartificial matrices of this invention can be pre-seeded with cells, whereby the cells are suspended in the matrix and exposed to the appropriate molecular cues in 3-D. These cell-seeded matrices are useful in tissue replacement protocols. According to these embodiments, tissue can be reconstituted in vitro and them implanted into a host in need thereof. For example, cardiac myoblasts may be suspended in the derivatized hydrogel matrices of this invention to create a tissue patch of a thickness corresponding to the cardiac wall. The reconstituted cardiac patch could then be implanted, as part of a tissue replacement therapy.

Detailed Description Text (27):
Similar protocols for cartilage, tendon, bone, skin, nerve, blood vessels and other tissues are contemplated. The ability to cast hydrogels, e.g., agarose, into a variety of shapes, as well the ability to fabricate "permissive" gel concentrations enables the production of bioartificial matrices that can influence cell behavior in defined planes or through defined "tracts".

Detailed Description Text (31):
CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) has been shown to evoke only 30% of the maximal response obtained by laminin in chemotactic functions with melanoma cells (Graf. et al., Biochemistry, 26, pp. 6896-900 (1987)). Thus, the use of full length ECM molecules may elicit more significant cellular effects. However,

the use of minimal oligopeptides creates a more stringent substrate condition and facilitates the testing of the gel system without the potent biological effects of full length proteins eclipsing the gel's physical effects. This enables the development and testing of a system with a base physical structure to support cell viability and influence cell behavior. The hydrogel matrix can then be rendered progressively more permissive by the use of appropriate covalently-coupled cell adhesion or extracellular matrix molecules.

Detailed Description Text (33):
In another preferred embodiment, the compositions of this invention may be used in neural cell transplantation. The ability of biosynthetic hydrogels to organize, support and direct neurite extension from neural cells may also be useful for applications such as 3-D neural cell culture and nerve regeneration. The bioartificial extracellular matrices of this invention may potentially carry one or more of the several cell adhesion molecules that have been identified to play an important role in cell migration and neurite extension in the developing nervous system, including N-CAM and Ng-CAM (Crossin et al., Proc. Natl. Acad. Sci., 82, pp. 6942-46 (1985); Daniloff et al., J. Neurosci., 6, pp. 739-58 (1986)), tenascin (Wehrle et al., Development, 1990, pp. 401-15 (1990) and L1 (Nieke and Schachner, Differentiation, 30, pp. 141-51 (1985)). Among extracellular matrix glycoproteins, laminin has been shown to be one of the most potent inducers of neurite outgrowth in vitro. It is a component of the Schwann cell basal lamina and is thought to be involved in axonal regeneration in vivo (Baron-Van-Evercooren et al., J. Neurosci. Res., 8, pp. 179-83 (1983); Manthorpe et al., J. Cell. Biol., 97, pp. 1882-90 (1983); Rogers et al., Dev. Biol., 113, pp. 429-35 (1983).

Detailed Description Text (51):
The agarose hydrogel compositions of this invention may be useful in nerve guidance channels. Such nerve guidance channels are well known in the art. Synthetic guidance channels have been used as inert conduits providing axonal guidance, maintaining growth factors, and preventing scar tissue invasion. Permselective channels with a molecular weight cut-off of 50,000 daltons allowed regeneration of nerves in a mouse sciatic nerve model. The regenerated nerves were characterized by fine epineurium and high numbers of myelinated axons. Aebischer et al., "The Use Of A Semi-Permeable Tube As A Guidance Channel For A Transected Rabbit Optic Nerve", In Gash & Sladek [Eds] Progress in Brain Research, 78, pp. 599-603 (1988).

Detailed Description Text (54):
The nerve guidance channels of the present invention include an implantable, biocompatible tubular permselective membrane having openings to receive the severed nerve. The lumen of the membrane preferably has a diameter ranging from about 0.5 mm to about 2.0 cm, to permit the nerve to regenerate through it. The thickness of the membrane may range from about 0.05 to about 1.0 mm. In some embodiments the membrane has a molecular weight cut-off of about 100,000 daltons or less. The membrane is preferably impermeable to fibroblasts and other scar-forming connective tissue cells. Additionally, the membrane may be composed of a biodegradable material. An agarose matrix is disposed in the lumen of the nerve guidance channel. The agarose concentration should range between 0.5 to 1.25%, preferably 1.0%. The average gel pore radius can vary between 120 to 290 nm, and is most preferably approximately 150 nm.

Detailed Description Text (55):
The optimal concentration of agarose gel for use as a regeneration matrix will vary according to the intended use of the matrix. The optimal concentration for in vitro use may not be optimal for the in vivo milieu. Neurite outgrowth in agarose gels is strongly dependent upon the pore size of agarose gels. Syneresis at the channel mid-point could alter the pore size of agarose gels enough to inhibit regeneration and therefore result in the absence of nerve cable in the mid-portion of the regeneration nerve bundle. It is important to account and if possible, correct for syneresis of the gel at channel mid-point. This may be overcome by two strategies.

One, the use of more dilute agarose gels to fill the channels may accommodate syneresis in the middle and still retain the pore size of gel at the channel midpoint to ranges permissible for neurite extension. Second, the use of a rough inner membrane of the channel may serve to prevent the fibroblasts induced syneresis of the gel inside the guidance channel (Aebischer et al., Brain Research, 531, pp. 21-18 (1990)).

Detailed Description Text (60):
The nerve guidance channels of this invention may additionally be seeded with Schwann cells. Schwann cells resident in the peripheral nerve trunk play a crucial role in the regenerative process. Schwann cells seeded in permselective synthetic guidance channels support extensive peripheral nerve regeneration. Schwann cells secrete laminin, which possesses neurite-promoting activity in vitro. See, e.g., Aebischer et al., Brain Research, 454, pp. 179-87 (1988). The Schwann cells are preferably longitudinally oriented along the guidance channel. This can be achieved by thermal manipulation of the agarose gel to orient the pores longitudinally, using methods well known in the art.

Detailed Description Text (63):
Briefly, various polymers and polymer blends can be used to manufacture the nerve guidance channel. Polymeric membranes, forming the nerve guidance channel may include polyacrylates (including acrylic copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethane, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones, polyphosphazenes, polyacrylonitriles, poly (acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof.

Detailed Description Text (65):
The jacket may have a single skin (Type 1, 2), or a double skin (Type 4). A single-skinned hollow fiber may be produced by quenching only one of the surfaces of the polymer solution as it is co-extruded. A double-skinned hollow fiber may be produced by quenching both surfaces o the polymer solution as it is co-extruded. Typically, a greater percentage of the outer surface of Type 1 hollow fibers is occupied by macropores compared to Type 4 hollow fibers. Type 2 hollow fibers are intermediate.

Detailed Description Text (66):
The jacket of the nerve guidance channel will have a pore size that determines the nominal molecular weight cut off (nMWCO) of the permselective membrane. Molecules larger than the nMWCO are physically impeded from traversing the membrane. Nominal molecular weight cut off is defined as 90% rejection under convective conditions. Typically the MWCO ranges between 50 and 200 kD, preferably between 50 and 100 kD.

Detailed Description Text (68):
In one embodiment, agarose hydrogels are used as a carried to present the laminin derived oligopeptide CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) to the site of nerve injury in an attempt to enhance nerve regeneration. Dorsal root ganglia have been shown to be responsive to CDPGYIGSR (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1) in vitro and show the greatest enhanced neuritic spread and neurite outgrowth compared to other fragments derived from laminin.

Detailed Description Text (73):
Characterization of Agarose Hydrogel Matrices

Detailed Description Text (83):
a. Hydraulic permeability: Gel blocks of different concentrations, each of thickness 0.5 cm and radius 1 cm, were mounted on a custom-built water column. Each block was subjected to a known hydraulic pressure, typically a 100 cm high H.sub.2 O column yielding approximately 24525 dynes/cm.sup.2. The hydraulic permeability per unit time for a given hydraulic pressure was measured for the various gel

concentrations. The average pore radius of the gel concentration range 0.5% to 5.0% was calculated as described by Refojo et al., J.Appl.Poly.Sci., 9, pp. 3417-26 (1965) using the hydraulic permeability.

Detailed Description Text (84):
The average pore radius, calculated from the hydraulic permeability measurements of the various agarose gels, decreased exponentially as the gel concentration increased (FIG. 1). E14 striatal cells did not extend neurities beyond a threshold agarose gel pore radius of 150 nm. The slope of the curve depicting pore radius was steep between gel concentrations of 1% and 2% indicating a strong dependence of pore size on gel concentration.

Detailed Description Text (85):
b. Scanning Electron microscopy (SEM): Agarose gels in the range 0.5% to 2.0% were freeze-dried, mounted on aluminum stubs, coated with gold and analyzed under a Joel 35M scanning electron microscope. Representative sections of the scanning electron micrographs were selected for evaluating the morphology and size of the pores.

Detailed Description Text (87):
c. Electron microscopy (ESEM): Agarose gels of the concentration range 0.5% to 2.5% were analyzed with an environmental scanning electron micrograph (Electroscan ESEM, type E3) under partially hydrated states to qualitatively asses gel pore morphology.

Detailed Description Text (88):
A decline in gel cavity radius was noted with increasing gel concentration. However, the nature and quality of images obtained with the ESEM allowed only qualitative conclusions on gel pore size to be drawn with confidence.

Detailed Description Text (89):
d. Gel electrophoresis: The electrophoretic mobility of insulin (Mw 5,700), bovine serum albumin (Mw. 66,000; radius 140 Angstroms) and bovine thyroglobulin (Mw. 669,000) in 1%, 2% and 4% agarose gels was measured under a constant electrophoretic voltage gradient. Twenty ml of the appropriate agarose gel concentration was poured into a DANAPHOR model 100 mini gel electrophoresis apparatus (Tectate S. S., Switzerland) with platinum electrodes. The proteins insulin, albumin and thyroglobulin were then subjected to a constant electrophoretic voltage gradient of 1 to 12V. The protein electrophoretic mobility was measured in the 1%, 2% and 4% agarose gels by measuring distance traveled per unit time. The relative electrophoretic velocity was then calculated after taking into account the isoelectric points of the different proteins, the voltage employed and the time of exposure to enable electrophoretic mobility comparisons of insulin, albumin thyroglobulin in the agarose gels.

Detailed Description Text (102):
The peptides used were GRGDSP (GlyArgGlyAspSerPro; SEQ ID NO:2) (Telios pharmaceuticals, San Diego, Calif.), CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1), the 19-mer sequence CSRARKQAASIKVAVSADR (CysSerArg AlaArgLysGlnAlaAlaSerIleLysValAlaValSerAlaAspArg; SEQ ID NO:3), and x-IKVAV-x containing sequence (s-IleLysValAlaVal-x; AA.sub.11 -AA.sub.15 of SEQ ID NO:3) (Anawa, Wagen, Switzerland) and as a control, GGGGG (GlyGlyGlyGlyGly; SEQ ID NO:4) (Sigma). A cocktail of the three aforementioned peptides (PEPMIX) was also immobilized to the hydrogel backbone at a concentration of 2 mg each in a total of 5 ml buffer solution.

Detailed Description Text (108):
Gel porosity of underivatized agarose gels and glycine coupled agarose was determined as described in Example 1. The average pore radius of the gels were determined to be 310 nm for a 0.5% underivatized agarose gel and 360 nm for a 0.5% glycine coupled agarose gel using the water column for hydraulic permeability

measurements.

Detailed Description Text (112):
Agarose hydrogels supported neurite outgrowth from DRGs in both X-Y and X-Z planes, demonstrating the 3-D character of neurite outgrowth in agarose gels. Fluorescein diacetate assay showed viable DRG neurons after 6 days in culture in all agarose gels used.

Detailed Description Text (122):
The effect of derivatized agarose gels on the regeneration of transected rat spinal dorsal roots was evaluated by using 6 mm long polymer guidance channels filled with CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1)--agarose to bridge a 4 mm gap in a transected dorsal root model. After 4 weeks, significantly greater numbers of myelinated axons were observed in the channels filled with CDPGYIGSR (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1)--agarose gels compared to channels filled with underivatized agarose gels.

Detailed Description Text (133):
Semi-thin cross-sections along the length of the guidance channel showed that myelinated axons were present all along the 4 mm nerve gap. Histological sections of guidance channels filled with agarose gels carrying regenerated dorsal roots showed doughnut shaped, centrally located nerve cables. Tissue reaction to the PAN/PVC polymer consisted of multi-nucleate giant cell and connective tissue infiltration. Neovascularisation was evident in the midst of regenerated nervous tissue along the Schwann cell infiltration. Light micrographs of regenerating dorsal root cables at 4 weeks post-implantation at the mid-point of guidance channels showed comparatively more nervous tissue in channels containing CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) gels relative to channels with underivatized agarose. At the mide-point of the 4 mm nerve gap, the channel filled with AgCDPGYIGSR (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1) had a significantly greater (p-0.05) number of myelinated axons compared to the channels with agarose plain gels (see FIG. 7). The number of myelinated axons at the midpoint in the agarose plain filled channels were comparable to those in saline filled channels described by McCormack et al., J.Comp. Neurol., 313, pp. 449-56 (1991). CDPGYIGSR-derivatized (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1) agarose gels had a significantly higher density ($p<0.05$) of myelinated axons at 0.5 mm and 2.0 mm along the channel at 4 weeks postimplantation (FIG. 8). The density of myelinated axons is defined as the number of myelinated axons per 10.sup.5 square microns of cable area.

Detailed Description Text (135):
All of the 10 mm long guidance channels implanted to bridge transected sural nerves were kinked due to flexion at the rats' knee-joint. Histological evaluation of nerves proximal to the kink showed regenerated cables located at the center of the channel with myelinated axons. All cables present in the channel distal to the kink contained a fibrotic cable but no myelinated axons. Almost all of the kinks occurred between 2-4 mm into the nerve gap. Therefore only the 2 mm point was analyzed for myelinated axons in this study. Light micrographs of regenerating sural nerves, 4 weeks post-implantation at the 2.0 mm point in polymer guidance channels filled Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) showed relatively higher numbers of nerve fascicles and neovascularization than in the other guidance channels. At 2 mm into the 8 mm nerve gap, guidance channels filled with AgCDPGYIGSR (CysAspPro GlyTyrIleGlySerArg; SEQ ID NO:1) gels had a significantly greater number ($p<0.05$) of myelinated axons compared to either the saline filled channels or agarose plain filled channels. See FIG. 9. At this point, the density of myelinated axons in the CDPGYIGSR-derivatized (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) agarose channels was also significantly greater ($p<0.05$) than the density of axons in the agarose-plain and saline-filled channels (FIG. 10). When the number of myelinated axons was compared to the number present in a normal control sural nerve, at 4 weeks, the average number of

myelinated axons regenerated across the saline filled channel was 12% of control sural nerve, 13% of control nerve for AgPlain filled channels and 40% of control sural nerve for AgCDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) filled channels.

Detailed Description Text (141):
This data indicates the feasibility of developing a matrix designed to enhance nerve regeneration by coupling neurite promoting biomolecules to agarose hydrogels.

Other Reference Publication (7):
Aebischer, P., et al., "Macroencapsulation of Dopamine-Secreting Cells By Coextrusion With an Organic Polymer Solution," Biomaterials, 12, pp. 50-56 (1991).

Other Reference Publication (21):
Chu, C.H., and A.M. Tolkovsky, "Alternative Adrenal Chromaffin Cell Fates Induced by Basic Fibroblast Growth Factor or Cyclic AMP In Vitro Depend on a Collaboration With The Growth Substrate," Neuroscience, 59, pp. 43-54 (1994).

Other Reference Publication (34):
Friedlander, David R., et al., "Functional Mapping of Cytotactin: Proteolytic Fragments Active in Cell-Substrate Adhesion," J. Cell Biol., 107, pp. 2329-2340 (1988).

Other Reference Publication (62):
Massia, Stephen P., and Jeffrey A. Hubbell, "Covalent Surface Immobilization of Arg-Gly-Asp- and Try-IIe-Gly-Ser-Arg-Containing Peptides to Obtain Well-Defined Cell-Adhesive Substrates," Analytical Biochemistry, 187, pp. 292-301 (1990).

Other Reference Publication (77):
Refojo, Miguel F., "Permeation of Water Through Some Hydrogels," J. Applied Polymer Science, 9, pp. 3417-3426 (1965).

Other Reference Publication (85):
Smalheiser, Neil R., et al., "Laminin As Substrate for Retinal Axons In Vitro," Dev. Brain Research, 12, pp. 136-140 (1984).

Other Reference Publication (94):
Woerly, S., et al., "Synthetic Polymer Matrices for Neural Cell Transplantation," Cell Transplantation, 2, pp. 229-239 (1993).

CLAIMS:

1. A bioartificial extracellular matrix comprising a three-dimensional high water content derivatized hydrogel matrix having a hydrogel matrix core

(a) wherein the hydrogel matrix is derivatized through the matrix by covalent-immobilization of at least one cell adhesive peptide fragment, homogeneously dispersed throughout the hydrogel matrix, and

(b) wherein the hydrogel matrix has an average pore radius greater than 120 nm.

5. The matrix according to any one of claims 1 and 2-4, wherein the hydrogel matrix is a polysaccharide hydrogel matrix.

6. The matrix according to claim 1, wherein the hydrogel matrix is an agarose hydrogel matrix.

7. The matrix according to claim 6, wherein the agarose concentration in the hydrogel matrix ranges between 0.5-1.25% (w/v) and the hydrogel matrix has an

average pore radius ranging between 120-290 nm.

8. The matrix according to claim 6, wherein the agarose concentration in the hydrogel matrix is 1.0% (w/v) and the hydrogel matrix has an average pore radius of approximately 150 nm.

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DOCUMENT-IDENTIFIER: US 6156572 A
TITLE: Bioartificial extracellular matrix containing hydrogel matrix derivatized with cell adhesive peptide fragment

Abstract Text (1):
A bioartificial extracellular matrix for use in tissue regeneration or replacement is provided by derivatizing a three-dimensional hydrogel matrix with a cell adhesive extracellular matrix protein or cell adhesive peptide fragment of the protein. Preferably, derivatizing is by covalent immobilization of a cell adhesive peptide fragment having the amino acid sequence, ArgGlyAsp, TyrIleGlySerArg or IleLysValAlaVal. Cartilage or tendon can be regenerated by implanting a matrix containing an adhesive peptide fragment that favors chondrocyte invasion. The matrix can be pre-seeded with cells, and tissue can be reconstituted in vitro and then implanted. A cell-seeded matrix can be encapsulated in a semi-permeable membrane to form a bioartificial organ. An agarose hydrogel matrix having an agarose concentration of 0.5-1.25% (w/v) and an average pore radius between 120 nm and 290 nm is preferred. A nerve guidance channel for use in regenerating severed nerve is prepared containing a tubular semi-permeable membrane having openings adapted to receive ends of a severed nerve, and an inner lumen containing the hydrogel matrix having a bound cell adhesive peptide fragment through which the nerve can regenerate.

Brief Summary Text (13):
All of the studies using these preptidic sequences of cell attachment and neurite promotion were conducted on flat two-dimension substrates (Smallheiser et al., Dev. Brain Res., 12, pp. 136-40 (1984); Graf et al., Biochemistry, 26, pp. 6896-900 (1987); Sephel et al., Biochem. Biophys. Res. Comm., 2, pp. 821-29 (1989); Jucker et al., J. Neurosci. Res., 28, pp. 507-17 (1991)). The physical and chemical nature of the culture substrate influences cell attachment and neurite extension. The physical microstructure of a 2-D culture substrate can influence cell behavior. The use of permissive and on-permissive culture surface chemistries facilitates nerve guidance in 2-D. The cell attachment regulating function of various serum proteins like albumin and fibronectin is dependent on the chemistries of the culture substrates that they are adsorbed onto.

Brief Summary Text (14):
Gene expression is reported to be regulated differently by a flat 2-D substrate as opposed to a hydrated 3-D substrate. For example, monolayer culture of primary rabbit articular chondrocyte and human epiphyseal chondrocyte on 2-D tissue culture substrates causes primary chondrocyte to lose their differentiated phenotype. The differentiated chondrocyte phenotype is re-expressed when they are cultured in 3-D agarose gels (Benya and Shaffer, Cell, 30, pp. 215-24 (1982); Aulthouse, et al., In Vitro Cell Dev. Bio., 25, pp. 659-68 (1989)).

Brief Summary Text (19):
This invention provides a three-dimensional hydrogel based, biosynthetic, extracellular matrix (ECM) equivalent, and method of making same. Agarose matrices having a chemistry amenable to derivatization with various ECM adhesive peptides and proteins, are preferred in forming the 3-D hydrogel substrates of this invention. These biologically active 3-D templates may be useful in facilitating

tissue regeneration or replacement.

Drawing Description Text (2):
FIG. 1. A double Y-axis plot depicting the influence of agarose gel concentration on average pore radius (Y1) and percent striatal cells extending neurites (Y2) after 72 hours in culture. Pore radius was calculated by hydraulic permeability measurements of the different gel concentrations. Solid line through pore radii data points is an exponential fit with r.sup.2 =0.985.

Drawing Description Text (8):
FIG. 7. Graph showing the number of myelinated axons regenerated at 4 weeks along polymer guidance channels filled with AgPlain and Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) gels. "*" depicts a statistically significant difference with p<0.05 using the Student t test.

Drawing Description Text (9):
FIG. 8. Graph showing the density of myelinated axons regenerated at 4 weeks along polymer guidance channels filled with AgPlain and Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) gels. "*" depicts a statistically significant difference with p<0.05 using the Student t test.

Drawing Description Text (10):
FIG. 9. Histogram depicting the number of myelinated axons in regenerating sural nerves at 2.0 mm distance from the proximal nerve stump in polymer guidance channels filled with A) saline; B) AgPlain and C) Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1). "*" depicts a statistical difference of p<0.05 when compared to saline or AgPlain. Student t test was used to evaluate statistical significance.

Drawing Description Text (11):
FIG. 10. Histogram depicting the density of myelinated axons in regenerating sural nerves at 2.0 mm distance from the proximal nerve stump in polymer guidance channels filled with A) saline; B) AgPlain and C) Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1). "*" depicts a statistical difference of p<0.05 when compared to saline or AgPlain. Student t test was used to evaluate statistical significance.

Detailed Description Text (2):
This invention provides a biosynthetic, hydrogel-based, three-dimensional bioartificial ECM. The bioartificial extracell lar matrices of this invention offer the possibility of manipulating cells in 3-D, and may be used as three dimensional templates for tissue engineering efforts in vitro and in vivo.

Detailed Description Text (5):
Any suitable hydrogel may be used as the substrate for the bioartificial extracellular matrices of this invention. Compositions that form hydrogels fall into three classes. The first class carries a net negative charge (e.g., alginate). The second class carries a net positive charge (e.g., collagen and laminin). Examples of commercially available extracellular matrix component hydrogels include Matrigel.TM. and Vitrogen.TM.. The third class is net neutral in charge (e.g., highly crosslinked polyethylene oxide, or polyvinylalcohol).

Detailed Description Text (6):
A hydrogel suitable for use in this invention is preferably a defined polymer, most preferably a polymer that is synthetic or can be prepared from a naturally occurring, non-tumorigenic source, free of undesired biological (e.g., bacterial or viral), chemical or other contaminants. Most preferred as the matrix substrate are well characterized hydrogels that permit presentation of only the desired ECM adhesion molecule or adhesive peptide fragement in 3-D, substantially free of undesired adhesion motifs.

Detailed Description Text (7):
Matrigel.TM. is not a defined substrate and also less desirable since it is derived from a murine sarcoma line. In addition, not all synthetic polymer hydrogels are suitable. For example, the use of acrylic based hydrogels by Woerly et al., Cell Transplantation, 2, pp. 229--39 (1993) presents the possibility of cytotoxicity because entrapment of neuronal cells is done concomitantly with the cross-linking reaction in the presence of free radical initiators.

Detailed Description Text (8):
Polymers that may be useful hydrogel matrix substrate materials include high molecular weight polyethylene oxide (PEO) and hyaluronate. Stabilized hyaluronate is commercially available (Fidia Advanced Biopolymers). Various PEO polymers are also commercially available.

Detailed Description Text (9):
Polysaccharides are a class of macromolecules of the general formula (CH.sub.2 O).sub.n which are useful as the hydrogel substrate in the present invention. Polysaccharides include several naturally occuring compounds, e.g., agarose, alginate and chitosan. We prefer agarose.

Detailed Description Text (10):
Agarose is a clear, thermoreversible hydrogel made of polysaccharides, mainly the alternating copolymers of 1,4 linked and 3,6-anhydro-.alpha.-L-galactose and 1,3 linked .beta.-D-galactose. Two commercially available agaroses are SeaPrep.RTM. and SeaPlaque.RTM. agarose (FMC Corp. Rockland, Me.). SeaPrep.RTM. is a hydroxyethylated agarose that gels at 17.degree. C. The particular suitability of a hydrogel as a biomaterial stems from the similarity of its physical properties to those of living tissues. This resemblance is based on its high water content, soft rubbery consistency and low interfacial tension. The thermoreversible properties of agarose gels make it possible for agarose to be a liquid at room temperature allowing for easy mixing of cell-gel solution and then cooling to 4.degree. C. causes gelation and entrapment of cells. This is a comparatively benign process, free of chemicals toxic to the cells.

Detailed Description Text (11):
We prefer an agarose concentration of 0.50 to 1.25% (w/v), most preferably 1.0%, for the permissive layers of the hydrogel matrix.

Detailed Description Text (12):
Several physical properties of the hydrogel matrices of this invention are dependent upon gel concentration. Increase in gel concentration may change the gel pore radius, morphology, or its permeability to different molecular weight proteins.

Detailed Description Text (13):
Gel pore radius determination can be determined by any suitable method, including hydraulic permeability determination using a graduated water column, transmission electron microscopy and sieving spheres of known radius through different agar gel concentrations. See, e.g., Griess et al., Biophysical J., 65, pp. 138-48 (1993). We prefer hydraulic permeability-based pore radius determination, as the method most sensitive to changes in gel concentration.

Detailed Description Text (14):
Measurement of gel hydraulic permeability using a graduated water column enabled the calculation of average pore radius for each of the gel concentrations studied. The average gel pore radius fall exponentially as the gel concentration increased. The slope of the curve indicated the sensitivity of pore radius to gel concentration. The average gel pore radius preferably varies between 120-290 nm, and is most preferably approximately 150 nm. The pore radius of the 1.25% threshold

agarose gel concentration was 150 nm.

Detailed Description Text (15):
The agarose hydrogels of this invention may be used as a carrier to present various ECM proteins or peptides, e.g., laminin fibronectin, and/or their peptidic analogs in 3-D. The chemistry of agarose permits easy modification with such ECM adhesive proteins and/or peptides. We prefer covalent immobilization of ECM proteins to the hydrogel backbone. Such immobilization is important because the physical blending of low molecular weight oligopeptides with hydrogels will not retain the peptides in the gel. Further, covalent immobilization prevents the possible saturation of cell surface receptors by `free-floating` ECM molecules in hydrogel-ECM molecule blends.

Detailed Description Text (17):
The bioartificial hydrogel extracellular matrices of this invention are useful for presenting in 3-D full length extracellular matrix proteins involved in cell adhesion. In addition, peptide fragments of such adhesion molecules that contain cell binding sequences may also be used (i.e., adhesive peptide fragement). Several such adhesive peptide fragments are known in the art. A particular peptide fragment can be tested for its binding ability or adhesive capacity according to standard techniques.

Detailed Description Text (18):
The bioartificial hydrogel matrices of this invention can be used to present ECM adhesion molecules, or adhesive peptide fragments thereof, in 3-D to a variety of cell types. These cell types include any cell that is normally in contact with the ECM in vivo, or any cell bearing a cell surface receptor capable of binding to an ECM adhesion molecule or adhesive peptide fragment thereof.

Detailed Description Text (24):
In some embodiments, the hydrogel ECM matrix can be derivatized with the appropriate ECM adhesion molecules or adhesive peptide fragments and implanted into a desired location in a host, e.g., a mammal, preferably a human. In these embodiments, the matrix acts as a support for tissue regeneration, whereby the host cells infiltrate the matrix. In the presence of the appropriate 3-D molecular cues in the matrix host tissue regeneration is facilitated.

Detailed Description Text (26):
In other embodiments, the bioartificial matrices of this invention can be pre-seeded with cells, whereby the cells are suspended in the matrix and exposed to the appropriate molecular cues in 3-D. These cell-seeded matrices are useful in tissue replacement protocols. According to these embodiments, tissue can be reconstituted in vitro and them implanted into a host in need thereof. For example, cardiac myoblasts may be suspended in the derivatized hydrogel matrices of this invention to create a tissue patch of a thickness corresponding to the cardiac wall. The reconstituted cardiac patch could then be implanted, as part of a tissue replacement therapy.

Detailed Description Text (27):
Similar protocols for cartilage, tendon, bone, skin, nerve, blood vessels and other tissues are contemplated. The ability to cast hydrogels, e.g., agarose, into a variety of shapes, as well the ability to fabricate "permissive" gel concentrations enables the production of bioartificial matrices that can influence cell behavior in defined planes or through defined "tracts".

Detailed Description Text (31):
CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) has been shown to evoke only 30% of the maximal response obtained by laminin in chemotactic functions with melanoma cells (Graf. et al., Biochemistry, 26, pp. 6896-900 (1987)). Thus, the use of full length ECM molecules may elicit more significant cellular effects. However,

the use of minimal oligopeptides creates a more stringent substrate condition and facilitates the testing of the gel system without the potent biological effects of full length proteins eclipsing the gel's physical effects. This enables the development and testing of a system with a base physical structure to support cell viability and influence cell behavior. The hydrogel matrix can then be rendered progressively more permissive by the use of appropriate covalently-coupled cell adhesion or extracellular matrix molecules.

Detailed Description Text (33):
In another preferred embodiment, the compositions of this invention may be used in neural cell transplantation. The ability of biosynthetic hydrogels to organize, support and direct neurite extension from neural cells may also be useful for applications such as 3-D neural cell culture and nerve regeneration. The bioartificial extracellular matrices of this invention may potentially carry one or more of the several cell adhesion molecules that have been identified to play an important role in cell migration and neurite extension in the developing nervous system, including N-CAM and Ng-CAM (Crossin et al., Proc. Natl. Acad. Sci., 82, pp. 6942-46 (1985); Daniloff et al., J. Neurosci., 6, pp. 739-58 (1986)), tenascin (Wehrle et al., Development, 1990, pp. 401-15 (1990) and L1 (Nieke and Schachner, Differentiation, 30, pp. 141-51 (1985)). Among extracellular matrix glycoproteins, laminin has been shown to be one of the most potent inducers of neurite outgrowth in vitro. It is a component of the Schwann cell basal lamina and is thought to be involved in axonal regeneration in vivo (Baron-Van-Evercooren et al., J. Neurosci. Res., 8, pp. 179-83 (1983); Manthorpe et al., J. Cell. Biol., 97, pp. 1882-90 (1983); Rogers et al., Dev. Biol., 113, pp. 429-35 (1983).

Detailed Description Text (51):
The agarose hydrogel compositions of this invention may be useful in nerve guidance channels. Such nerve guidance channels are well known in the art. Synthetic guidance channels have been used as inert conduits providing axonal guidance, maintaining growth factors, and preventing scar tissue invasion. Permselective channels with a molecular weight cut-off of 50,000 daltons allowed regeneration of nerves in a mouse sciatic nerve model. The regenerated nerves were characterized by fine epineurium and high numbers of myelinated axons. Aebischer et al., "The Use Of A Semi-Permeable Tube As A Guidance Channel For A Transected Rabbit Optic Nerve", In Gash & Sladek [Eds] Progress in Brain Research, 78, pp. 599-603 (1988).

Detailed Description Text (54):
The nerve guidance channels of the present invention include an implantable, biocompatible tubular permselective membrane having openings to receive the severed nerve. The lumen of the membrane preferably has a diameter ranging from about 0.5 mm to about 2.0 cm, to permit the nerve to regenerate through it. The thickness of the membrane may range from about 0.05 to about 1.0 mm. In some embodiments the membrane has a molecular weight cut-off of about 100,000 daltons or less. The membrane is preferably impermeable to fibroblasts and other scar-forming connective tissue cells. Additionally, the membrane may be composed of a biodegradable material. An agarose matrix is disposed in the lumen of the nerve guidance channel. The agarose concentration should range between 0.5 to 1.25%, preferably 1.0%. The average gel pore radius can vary between 120 to 290 nm, and is most preferably approximately 150 nm.

Detailed Description Text (55):
The optimal concentration of agarose gel for use as a regeneration matrix will vary according to the intended use of the matrix. The optimal concentration for in vitro use may not be optimal for the in vivo milieu. Neurite outgrowth in agarose gels is strongly dependent upon the pore size of agarose gels. Syneresis at the channel mid-point could alter the pore size of agarose gels enough to inhibit regeneration and therefore result in the absence of nerve cable in the mid-portion of the regeneration nerve bundle. It is important to account and if possible, correct for syneresis of the gel at channel mid-point. This may be overcome by two strategies.

One, the use of more dilute agarose gels to fill the channels may accommodate syneresis in the middle and still retain the pore size of gel at the channel midpoint to ranges permissible for neurite extension. Second, the use of a rough inner membrane of the channel may serve to prevent the fibroblasts induced syneresis of the gel inside the guidance channel (Aebischer et al., Brain Research, 531, pp. 21-18 (1990)).

Detailed Description Text (60):
The nerve guidance channels of this invention may additionally be seeded with Schwann cells. Schwann cells resident in the peripheral nerve trunk play a crucial role in the regenerative process. Schwann cells seeded in permselective synthetic guidance channels support extensive peripheral nerve regeneration. Schwann cells secrete laminin, which possesses neurite-promoting activity in vitro. See, e.g., Aebischer et al., Brain Research, 454, pp. 179-87 (1988). The Schwann cells are preferably longitudinally oriented along the guidance channel. This can be achieved by thermal manipulation of the agarose gel to orient the pores longitudinally, using methods well known in the art.

Detailed Description Text (63):
Briefly, various polymers and polymer blends can be used to manufacture the nerve guidance channel. Polymeric membranes, forming the nerve guidance channel may include polyacrylates (including acrylic copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethane, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones, polyphosphazenes, polyacrylonitriles, poly (acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof.

Detailed Description Text (65):
The jacket may have a single skin (Type 1, 2), or a double skin (Type 4). A single-skinned hollow fiber may be produced by quenching only one of the surfaces of the polymer solution as it is co-extruded. A double-skinned hollow fiber may be produced by quenching both surfaces o the polymer solution as it is co-extruded. Typically, a greater percentage of the outer surface of Type 1 hollow fibers is occupied by macropores compared to Type 4 hollow fibers. Type 2 hollow fibers are intermediate.

Detailed Description Text (66):
The jacket of the nerve guidance channel will have a pore size that determines the nominal molecular weight cut off (nMWCO) of the permselective membrane. Molecules larger than the nMWCO are physically impeded from traversing the membra e. Nominal molecular weight cut off is defined as 90% rejection under convective conditions. Typically the MWCO ranges between 50 and 200 kD, preferably between 50 and 100 kD.

Detailed Description Text (68):
In one embodiment, agarose hydrogels are used as a carried to present the laminin derived oligopeptide CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) to the site of nerve injury in an attempt to enhance nerve regeneration. Dorsal root ganglia have been shown to be responsive to CDPGYIGSR (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1) in vitro and show the greatest enhanced neuritic spread and neurite outgrowth compared to other fragments derived from laminin.

Detailed Description Text (73):
Characterization of Agarose Hydrogel Matrices

Detailed Description Text (83):
a. Hydraulic permeability: Gel blocks of different concentrations, each of thickness 0.5 cm and radius 1 cm, were mounted on a custom-built water column. Each block was subjected to a known hydraulic pressure, typically a 100 cm high H.sub.2 O column yielding approximately 24525 dynes/cm.sup.2. The hydraulic permeability per unit time for a given hydraulic pressure was measured for the various gel

concentrations. The average pore radius of the gel concentration range 0.5% to 5.0% was calculated as described by Refojo et al., J.Appl.Poly.Sci., 9, pp. 3417-26 (1965) using the hydraulic permeability.

Detailed Description Text (84):
The average pore radius, calculated from the hydraulic permeability measurements of the various agarose gels, decreased exponentially as the gel concentration increased (FIG. 1). E14 striatal cells did not extend neurities beyond a threshold agarose gel pore radius of 150 nm. The slope of the curve depicting pore radius was steep between gel concentrations of 1% and 2% indicating a strong dependence of pore size on gel concentration.

Detailed Description Text (85):
b. Scanning Electron microscopy (SEM): Agarose gels in the range 0.5% to 2.0% were freeze-dried, mounted on aluminum stubs, coated with gold and analyzed under a Joel 35M scanning electron microscope. Representative sections of the scanning electron micrographs were selected for evaluating the morphology and size of the pores.

Detailed Description Text (87):
c. Electron microscopy (ESEM): Agarose gels of the concentration range 0.5% to 2.5% were analyzed with an environmental scanning electron micrograph (Electroscan ESEM, type E3) under partially hydrated states to qualitatively asses gel pore morphology.

Detailed Description Text (88):
A decline in gel cavity radius was noted with increasing gel concentration. However, the nature and quality of images obtained with the ESEM allowed only qualitative conclusions on gel pore size to be drawn with c nfidence.

Detailed Description Text (89):
d. Gel electrophoresis: The electrophoretic mobility of insulin (Mw 5,700), bovine serum albumin (Mw. 66,000; radius 140 Angstroms) and bovine thyroglobulin (Mw. 669,000) in 1%, 2% and 4% agarose gels was measured under a constant electrophoretic voltage gradient. Twenty ml of the appropriate agarose gel concentration was poured into a DANAPHOR model 100 mini gel electrophoresis apparatus (Tectate S. S., Switzerland) with platinum electrodes. The proteins insulin, albumin and thyroglobulin were then subjected to a constant electrophoretic voltage gradient of 1 to 12V. The protein electrophoretic mobility was measured in the 1%, 2% and 4% agarose gels by measuring distance traveled per unit time. The relative electrophoretic velocity was then calculated after taking into account the isoelectric points of the different proteins, the voltage employed and the time of exposure to enable electrophoretic mobility comparisons of insulin, albumin thyroglobulin in the agarose gels.

Detailed Description Text (102):
The peptides used were GRGDSP (GlyArgGlyAspSerPro; SEQ ID NO:2) (Telios pharmaceuticals, San Diego, Calif.), CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1), the 19-mer sequence CSRARKQAASIKVAVSADR (CysSerArg AlaArgLysGlnAlaAlaSerIleLysValAlaValSerAlaAspArg; SEQ ID NO:3), and x-IKVAV-x containing sequence (s-IleLysValAlaVal-x; AA.sub.11 -AA.sub.15 of SEQ ID NO:3) (Anawa, Wagen, Switzerland) and as a control, GGGGG (GlyGlyGlyGlyGly; SEQ ID NO:4) (Sigma). A cocktail of the three aforementioned peptides (PEPMIX) was also immobilized to the hydrogel backbone at a concentration of 2 mg each in a total of 5 ml buffer solution.

Detailed Description Text (108):
Gel porosity of underivatized agarose gels and glycine coupled agarose was determined as described in Example 1. The average pore radius of the gels were determined to be 310 nm for a 0.5% underivatized agarose gel and 360 nm for a 0.5% glycine coupled agarose gel using the water column for hydraulic permeability

measurements.

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Agarose hydrogels supported neurite outgrowth from DRGs in both X-Y and X-Z planes, demonstrating the 3-D character of neurite outgrowth in agarose gels. Fluorescein diacetate assay showed viable DRG neurons after 6 days in culture in all agarose gels used.

Detailed Description Text (122):
The effect of derivatized agarose gels on the regeneration of transected rat spinal dorsal roots was evaluated by using 6 mm long polymer guidance channels filled with CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1)--agarose to bridge a 4 mm gap in a transected dorsal root model. After 4 weeks, significantly greater numbers of myelinated axons were observed in the channels filled with CDPGYIGSR (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1)--agarose gels compared to channels filled with underivatized agarose gels.

Detailed Description Text (133):
Semi-thin cross-sections along the length of the guidance channel showed that myelinated axons were present all along the 4 mm nerve gap. Histological sections of guidance channels filled with agarose gels carrying regenerated dorsal roots showed doughnut shaped, centrally located nerve cables. Tissue reaction to the PAN/PVC polymer consisted of multi-nucleate giant cell and connective tissue infiltration. Neovascularisation was evident in the midst of regenerated nervous tissue along the Schwann cell infiltration. Light micrographs of regenerating dorsal root cables at 4 weeks post-implantation at the mid-point of guidance channels showed comparatively more nervous tissue in channels containing CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) gels relative to channels with underivatized agarose. At the mide-point of the 4 mm nerve gap, the channel filled with AgCDPGYIGSR (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1) had a significantly greater (p-0.05) number of myelinated axons compared to the channels with agarose plain gels (see FIG. 7). The number of myelinated axons at the midpoint in the agarose plain filled channels were comparable to those in saline filled channels described by McCormack et al., J.Comp. Neurol., 313, pp. 449-56 (1991). CDPGYIGSR-derivatized (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1) agarose gels had a significantly higher density ($p<0.05$) of myelinated axons at 0.5 mm and 2.0 mm along the channel at 4 weeks postimplantation (FIG. 8). The density of myelinated axons is defined as the number of myelinated axons per 10.sup.5 square microns of cable area.

Detailed Description Text (135):
All of the 10 mm long guidance channels implanted to bridge transected sural nerves were kinked due to flexion at the rats' knee-joint. Histological evaluation of nerves proximal to the kink showed regenerated cables located at the center of the channel with myelinated axons. All cables present in the channel distal to the kink contained a fibrotic cable but no myelinated axons. Almost all of the kinks occurred between 2-4 mm into the nerve gap. Therefore only the 2 mm point was analyzed for myelinated axons in this study. Light micrographs of regenerating sural nerves, 4 weeks post-implantation at the 2.0 mm point in polymer guidance channels filled Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) showed relatively higher numbers of nerve fascicles and eovascularization than in the other guidance channels. At 2 mm into the 8 mm nerve gap, guidance channels filled with AgCDPGYIGSR (CysAspPro GlyTyrIleGlySerArg; SEQ ID NO:1) gels had a significantly greater number ($p<0.05$) of myelinated axons compared to either the saline filled channels or agarose plain filled channels. See FIG. 9. At this point, the density of myelinated axons in the CDPGYIGSR-derivatized (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) agarose channels was also significantly greater ($p<0.05$) than the density of axons in the agarose-plain and saline-filled channels (FIG. 10). When the number of myelinated axons was compared to the number present in a normal control sural nerve, at 4 weeks, the average number of

myelinated axons regenerated across the saline filled channel was 12% of control sural nerve, 13% of control nerve for AgPlain filled channels and 40% of control sural nerve for AgCDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) filled channels.

Detailed Description Text (141):
This data indicates the feasibility of developing a matrix designed to enhance nerve regeneration by coupling neurite promoting biomolecules to agarose hydrogels.

Other Reference Publication (7):
Aebischer, P., et al., "Macroencapsulation of Dopamine-Secreting Cells By Coextrusion With an Organic Polymer Solution," Biomaterials, 12, pp. 50-56 (1991).

Other Reference Publication (21):
Chu, C.H., and A.M. Tolkovsky, "Alternative Adrenal Chromaffin Cell Fates Induced

With The Growth Substrate," Neuroscience, 59, pp. 43-54 (1994).

Other Reference Publication (34):
Friedlander, David R., et al., "Functional Mapping of Cytotactin: Proteolytic Fragments Active in Cell-Substrate Adhesion," J. Cell Biol., 107, pp. 2329-2340 (1988).

Other Reference Publication (62):
Massia, Stephen P., and Jeffrey A. Hubbell, "Covalent Surface Immobilization of Arg-Gly-Asp- and Try-IIe-Gly-Ser-Arg-Containing Peptides to Obtain Well-Defined Cell-Adhesive Substrates," Analytical Biochemistry, 187, pp. 292-301 (1990).

Other Reference Publication (77):
Refojo, Miguel F., "Permeation of Water Through Some Hydrogels," J. Applied Polymer Science, 9, pp. 3417-3426 (1965).

Other Reference Publication (85):
Smalheiser, Neil R., et al., "Laminin As Substrate for Retinal Axons In Vitro," Dev. Brain Research, 12, pp. 136-140 (1984).

Other Reference Publication (94):
Woerly, S., et al., "Synthetic Polymer Matrices for Neural Cell Transplantation," Cell Transplantation, 2, pp. 229-239 (1993).

CLAIMS:

1. A bioartificial extracellular matrix comprising a three-dimensional high water content derivatized hydrogel matrix having a hydrogel matrix core

(a) wherein the hydrogel matrix is derivatized through the matrix by covalent-immobilization of at least one cell adhesive peptide fragment, homogeneously dispersed throughout the hydrogel matrix, and

(b) wherein the hydrogel matrix has an average pore radius greater than 120 nm.

5. The matrix according to any one of claims 1 and 2-4, wherein the hydrogel matrix is a polysaccharide hydrogel matrix.

6. The matrix according to claim 1, wherein the hydrogel matrix is an agarose hydrogel matrix.

7. The matrix according to claim 6, wherein the agarose concentration in the hydrogel matrix ranges between 0.5-1.25% (w/v) and the hydrogel matrix has an

average pore radius ranging between 120-290 nm.

8. The matrix according to claim 6, wherein the agarose concentration in the hydrogel matrix is 1.0% (w/v) and the hydrogel matrix has an average pore radius of approximately 150 nm.

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DOCUMENT-IDENTIFIER: US 6156572 A
TITLE: Bioartificial extracellular matrix containing hydrogel matrix derivatized with cell adhesive peptide fragment

Abstract Text (1):
A bioartificial extracellular matrix for use in tissue regeneration or replacement is provided by derivatizing a three-dimensional hydrogel matrix with a cell adhesive extracellular matrix protein or cell adhesive peptide fragment of the protein. Preferably, derivatizing is by covalent immobilization of a cell adhesive peptide fragment having the amino acid sequence, ArgGlyAsp, TyrIleGlySerArg or IleLysValAlaVal. Cartilage or tendon can be regenerated by implanting a matrix containing an adhesive peptide fragment that favors chondrocyte invasion. The matrix can be pre-seeded with cells, and tissue can be reconstituted in vitro and then implanted. A cell-seeded matrix can be encapsulated in a semi-permeable membrane to form a bioartificial organ. An agarose hydrogel matrix having an agarose concentration of 0.5-1.25% (w/v) and an average pore radius between 120 nm and 290 nm is preferred. A nerve guidance channel for use in regenerating severed nerve is prepared containing a tubular semi-permeable membrane having openings adapted to receive ends of a severed nerve, and an inner lumen containing the hydrogel matrix having a bound cell adhesive peptide fragment through which the nerve can regenerate.

Brief Summary Text (13):
All of the studies using these preptidic sequences of cell attachment and neurite promotion were conducted on flat two-dimension substrates (Smallheiser et al., Dev. Brain Res., 12, pp. 136-40 (1984); Graf et al., Biochemistry, 26, pp. 6896-900 (1987); Sephel et al., Biochem. Biophys. Res. Comm., 2, pp. 821-29 (1989); Jucker et al., J. Neurosci. Res., 28, pp. 507-17 (1991)). The physical and chemical nature of the culture substrate influences cell attachment and neurite extension. The physical microstructure of a 2-D culture substrate can influence cell behavior. The use of permissive and on-permissive culture surface chemistries facilitates nerve guidance in 2-D. The cell attachment regulating function of various serum proteins like albumin and fibronectin is dependent on the chemistries of the culture substrates that they are adsorbed onto.

Brief Summary Text (14):
Gene expression is reported to be regulated differently by a flat 2-D substrate as opposed to a hydrated 3-D substrate. For example, monolayer culture of primary rabbit articular chondrocyte and human epiphyseal chondrocyte on 2-D tissue culture substrates causes primary chondrocyte to lose their differentiated phenotype. The differentiated chondrocyte phenotype is re-expressed when they are cultured in 3-D agarose gels (Benya and Shaffer, Cell, 30, pp. 215-24 (1982); Aulthouse, et al., In Vitro Cell Dev. Bio., 25, pp. 659-68 (1989)).

Brief Summary Text (19):
This invention provides a three-dimensional hydrogel based, biosynthetic, extracellular matrix (ECM) equivalent, and method of making same. Agarose matrices having a chemistry amenable to derivatization with various ECM adhesive peptides and proteins, are preferred in forming the 3-D hydrogel substrates of this invention. These biologically active 3-D templates may be useful in facilitating

tissue regeneration or replacement.

Drawing Description Text (2):
FIG. 1. A double Y-axis plot depicting the influence of agarose gel concentration on average pore radius (Y1) and percent striatal cells extending neurites (Y2) after 72 hours in culture. Pore radius was calculated by hydraulic permeability measurements of the different gel concentrations. Solid line through pore radii data points is an exponential fit with r.sup.2 =0.985.

Drawing Description Text (8):
FIG. 7. Graph showing the number of myelinated axons regenerated at 4 weeks along polymer guidance channels filled with AgPlain and Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) gels. "*" depicts a statistically significant difference with $p<0.05$ using the Student t test.

Drawing Description Text (9):
FIG. 8. Graph showing the density of myelinated axons regenerated at 4 weeks along polymer guidance channels filled with AgPlain and Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) gels. "*" depicts a statistically significant difference with $p<0.05$ using the Student t test.

Drawing Description Text (10):
FIG. 9. Histogram depicting the number of myelinated axons in regenerating sural nerves at 2.0 mm distance from the proximal nerve stump in polymer guidance channels filled with A) saline; B) AgPlain and C) Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1). "*" depicts a statistical difference of $p<0.05$ when compared to saline or AgPlain. Student t test was used to evaluate statistical significance.

Drawing Description Text (11):
FIG. 10. Histogram depicting the density of myelinated axons in regenerating sural nerves at 2.0 mm distance from the proximal nerve stump in polymer guidance channels filled with A) saline; B) AgPlain and C) Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1). "*" depicts a statistical difference of $p<0.05$ when compared to saline or AgPlain. Student t test was used to evaluate statistical significance.

Detailed Description Text (2):
This invention provides a biosynthetic, hydrogel-based, three-dimensional bioartificial ECM. The bioartificial extracellular matrices of this invention offer the possibility of manipulating cells in 3-D, and may be used as three dimensional templates for tissue engineering efforts in vitro and in vivo.

Detailed Description Text (5):
Any suitable hydrogel may be used as the substrate for the bioartificial extracellular matrices of this invention. Compositions that form hydrogels fall into three classes. The first class carries a net negative charge (e.g., alginate). The second class carries a net positive charge (e.g., collagen and laminin). Examples of commercially available extracellular matrix component hydrogels include Matrigel.TM. and Vitrogen.TM.. The third class is net neutral in charge (e.g., highly crosslinked polyethylene oxide, or polyvinylalcohol).

Detailed Description Text (6):
A hydrogel suitable for use in this invention is preferably a defined polymer, most preferably a polymer that is synthetic or can be prepared from a naturally occurring, non-tumorigenic source, free of undesired biological (e.g., bacterial or viral), chemical or other contaminants. Most preferred as the matrix substrate are well characterized hydrogels that permit presentation of only the desired ECM adhesion molecule or adhesive peptide fragement in 3-D, substantially free of undesired adhesion motifs.

Detailed Description Text (7):
Matrigel.TM. is not a defined substrate and also less desirable since it is derived from a murine sarcoma line. In addition, not all synthetic polymer hydrogels are suitable. For example, the use of acrylic based hydrogels by Woerly et al., Cell Transplantation, 2, pp. 229--39 (1993) presents the possibility of cytotoxicity because entrapment of neuronal cells is done concomitantly with the cross-linking reaction in the presence of free radical initiators.

Detailed Description Text (8):
Polymers that may be useful hydrogel matrix substrate materials include high molecular weight polyethylene oxide (PEO) and hyaluronate. Stabilized hyaluronate is commercially available (Fidia Advanced Biopolymers). Various PEO polymers are also commercially available.

Detailed Description Text (9):
Polysaccharides are a class of macromolecules of the general formula (CH.sub.2 O).sub.n which are useful as the hydrogel substrate in the present invention. Polysaccharides include several naturally occuring compounds, e.g., agarose, alginate and chitosan. We prefer agarose.

Detailed Description Text (10):
Agarose is a clear, thermoreversible hydrogel made of polysaccharides, mainly the alternating copolymers of 1,4 linked and 3,6-anhydro-.alpha.-L-galactose and 1,3 linked .beta.-D-galactose. Two commercially available agaroses are SeaPrep.RTM. and SeaPlaque.RTM. agarose (FMC Corp. Rockland, Me.). SeaPrep.RTM. is a hydroxyethylated agarose that gels at 17.degree. C. The particular suitability of a hydrogel as a biomaterial stems from the similarity of its physical properties to those of living tissues. This resemblance is based on its high water content, soft rubbery consistency and low interfacial tension. The thermoreversible properties of agarose gels make it possible for agarose to be a liquid at room temperature allowing for easy mixing of cell-gel solution and then cooling to 4.degree. C. causes gelation and entrapment of cells. This is a comparatively benign process, free of chemicals toxic to the cells.

Detailed Description Text (11):
We prefer an agarose concentration of 0.50 to 1.25% (w/v), most preferably 1.0%, for the permissive layers of the hydrogel matrix.

Detailed Description Text (12):
Several physical properties of the hydrogel matrices of this invention are dependent upon gel concentration. Increase in gel concentration may change the gel pore radius, morphology, or its permeability to different molecular weight proteins.

Detailed Description Text (13):
Gel pore radius determination can be determined by any suitable method, including hydraulic permeability determination using a graduated water column, transmission electron microscopy and sieving spheres of known radius through different agar gel concentrations. See, e.g., Griess et al., Biophysical J., 65, pp. 138-48 (1993). We prefer hydraulic permeability-based pore radius determination, as the method most sensitive to changes in gel concentration.

Detailed Description Text (14):
Measurement of gel hydraulic permeability using a graduated water column enabled the calculation of average pore radius for each of the gel concentrations studied. The average gel pore radius fall exponentially as the gel concentration increased. The slope of the curve indicated the sensitivity of pore radius to gel concentration. The average gel pore radius preferably varies between 120-290 nm, and is most preferably approximately 150 nm. The pore radius of the 1.25% threshold

agarose gel concentration was 150 nm.

Detailed Description Text (15):
The agarose hydrogels of this invention may be used as a carrier to present various ECM proteins or peptides, e.g., laminin fibronectin, and/or their peptidic analogs in 3-D. The chemistry of agarose permits easy modification with such ECM adhesive proteins and/or peptides. We prefer covalent immobilization of ECM proteins to the hydrogel backbone. Such immobilization is important because the physical blending of low molecular weight oligopeptides with hydrogels will not retain the peptides in the gel. Further, covalent immobilization prevents the possible saturation of cell surface receptors by `free-floating` ECM molecules in hydrogel-ECM molecule blends.

Detailed Description Text (17):
The bioartificial hydrogel extracellular matrices of this invention are useful for presenting in 3-D full length extracellular matrix proteins involved in cell adhesion. In addition, peptide fragments of such adhesion molecules that contain cell binding sequences may also be used (i.e., adhesive peptide fragement). Several such adhesive peptide fragments are known in the art. A particular peptide fragment can be tested for its binding ability or adhesive capacity according to standard techniques.

Detailed Description Text (18):
The bioartificial hydrogel matrices of this invention can be used to present ECM adhesion molecules, or adhesive peptide fragments thereof, in 3-D to a variety of cell types. These cell types include any cell that is normally in contact with the ECM in vivo, or any cell bearing a cell surface receptor capable of binding to an ECM adhesion molecule or adhesive peptide fragment thereof.

Detailed Description Text (24):
In some embodiments, the hydrogel ECM matrix can be derivatized with the appropriate ECM adhesion molecules or adhesive peptide fragments and implanted into a desired location in a host, e.g., a mammal, preferably a human. In these embodiments, the matrix acts as a support for tissue regeneration, whereby the host cells infiltrate the matrix. In the presence of the appropriate 3-D molecular cues in the matrix host tissue regeneration is facilitated.

Detailed Description Text (26):
In other embodiments, the bioartificial matrices of this invention can be pre-seeded with cells, whereby the cells are suspended in the matrix and exposed to the appropriate molecular cues in 3-D. These cell-seeded matrices are useful in tissue replacement protocols. According to these embodiments, tissue can be reconstituted in vitro and them implanted into a host in need thereof. For example, cardiac myoblasts may be suspended in the derivatized hydrogel matrices of this invention to create a tissue patch of a thickness corresponding to the cardiac wall. The reconstituted cardiac patch could then be implanted, as part of a tissue replacement therapy.

Detailed Description Text (27):
Similar protocols for cartilage, tendon, bone, skin, nerve, blood vessels and other tissues are contemplated. The ability to cast hydrogels, e.g., agarose, into a variety of shapes, as well the ability to fabricate "permissive" gel concentrations enables the production of bioartificial matrices that can influence cell behavior in defined planes or through defined "tracts".

Detailed Description Text (31):
CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) has been shown to evoke only 30% of the maximal response obtained by laminin in chemotactic functions with melanoma cells (Graf. et al., Biochemistry, 26, pp. 6896-900 (1987)). Thus, the use of full length ECM molecules may elicit more significant cellular effects. However,

the use of minimal oligopeptides creates a more stringent substrate condition and facilitates the testing of the gel system without the potent biological effects of full length proteins eclipsing the gel's physical effects. This enables the development and testing of a system with a base physical structure to support cell viability and influence cell behavior. The hydrogel matrix can then be rendered progressively more permissive by the use of appropriate covalently-coupled cell adhesion or extracellular matrix molecules.

Detailed Description Text (33):
In another preferred embodiment, the compositions of this invention may be used in neural cell transplantation. The ability of biosynthetic hydrogels to organize, support and direct neurite extension from neural cells may also be useful for applications such as 3-D neural cell culture and nerve regeneration. The bioartificial extracellular matrices of this invention may potentially carry one or more of the several cell adhesion molecules that have been identified to play an important role in cell migration and neurite extension in the developing nervous system, including N-CAM and Ng-CAM (Crossin et al., Proc. Natl. Acad. Sci., 82, pp. 6942-46 (1985); Daniloff et al., J. Neurosci., 6, pp. 739-58 (1986)), tenascin (Wehrle et al., Development, 1990, pp. 401-15 (1990) and L1 (Nieke and Schachner, Differentiation, 30, pp. 141-51 (1985)). Among extracellular matrix glycoproteins, laminin has been shown to be one of the most potent inducers of neurite outgrowth in vitro. It is a component of the Schwann cell basal lamina and is thought to be involved in axonal regeneration in vivo (Baron-Van-Evercooren et al., J. Neurosci. Res., 8, pp. 179-83 (1983); Manthorpe et al., J. Cell. Biol., 97, pp. 1882-90 (1983); Rogers et al., Dev. Biol., 113, pp. 429-35 (1983).

Detailed Description Text (51):
The agarose hydrogel compositions of this invention may be useful in nerve guidance channels. Such nerve guidance channels are well known in the art. Synthetic guidance channels have been used as inert conduits providing axonal guidance, maintaining growth factors, and preventing scar tissue invasion. Permselective channels with a molecular weight cut-off of 50,000 daltons allowed regeneration of nerves in a mouse sciatic nerve model. The regenerated nerves were characterized by fine epineurium and high numbers of myelinated axons. Aebischer et al., "The Use Of A Semi-Permeable Tube As A Guidance Channel For A Transected Rabbit Optic Nerve", In Gash & Sladek [Eds] Progress in Brain Research, 78, pp. 599-603 (1988).

Detailed Description Text (54):
The nerve guidance channels of the present invention include an implantable, biocompatible tubular permselective membrane having openings to receive the severed nerve. The lumen of the membrane preferably has a diameter ranging from about 0.5 mm to about 2.0 cm, to permit the nerve to regenerate through it. The thickness of the membrane may range from about 0.05 to about 1.0 mm. In some embodiments the membrane has a molecular weight cut-off of about 100,000 daltons or less. The membrane is preferably impermeable to fibroblasts and other scar-forming connective tissue cells. Additionally, the membrane may be composed of a biodegradable material. An agarose matrix is disposed in the lumen of the nerve guidance channel. The agarose concentration should range between 0.5 to 1.25%, preferably 1.0%. The average gel pore radius can vary between 120 to 290 nm, and is most preferably approximately 150 nm.

Detailed Description Text (55):
The optimal concentration of agarose gel for use as a regeneration matrix will vary according to the intended use of the matrix. The optimal concentration for in vitro use may not be optimal for the in vivo milieu. Neurite outgrowth in agarose gels is strongly dependent upon the pore size of agarose gels. Syneresis at the channel mid-point could alter the pore size of agarose gels enough to inhibit regeneration and therefore result in the absence of nerve cable in the mid-portion of the regeneration nerve bundle. It is important to account and if possible, correct for syneresis of the gel at channel mid-point. This may be overcome by two strategies.

One, the use of more dilute agarose gels to fill the channels may accommodate syneresis in the middle and still retain the pore size of gel at the channel midpoint to ranges permissible for neurite extension. Second, the use of a rough inner membrane of the channel may serve to prevent the fibroblasts induced syneresis of the gel inside the guidance channel (Aebischer et al., Brain Research, 531, pp. 21-18 (1990)).

Detailed Description Text (60):
The nerve guidance channels of this invention may additionally be seeded with Schwann cells. Schwann cells resident in the peripheral nerve trunk play a crucial role in the regenerative process. Schwann cells seeded in permselective synthetic guidance channels support extensive peripheral nerve regeneration. Schwann cells secrete laminin, which possesses neurite-promoting activity in vitro. See, e.g., Aebischer et al., Brain Research, 454, pp. 179-87 (1988). The Schwann cells are preferably longitudinally oriented along the guidance channel. This can be achieved by thermal manipulation of the agarose gel to orient the pores longitudinally, using methods well known in the art.

Detailed Description Text (63):
Briefly, various polymers and polymer blends can be used to manufacture the nerve guidance channel. Polymeric membranes, forming the nerve guidance channel may include polyacrylates (including acrylic copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethane, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones, polyphosphazenes, polyacrylonitriles, poly (acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof.

Detailed Description Text (65):
The jacket may have a single skin (Type 1, 2), or a double skin (Type 4). A single-skinned hollow fiber may be produced by quenching only one of the surfaces of the polymer solution as it is co-extruded. A double-skinned hollow fiber may be produced by quenching both surfaces o the polymer solution as it is co-extruded. Typically, a greater percentage of the outer surface of Type 1 hollow fibers is occupied by macropores compared to Type 4 hollow fibers. Type 2 hollow fibers are intermediate.

Detailed Description Text (66):
The jacket of the nerve guidance channel will have a pore size that determines the nominal molecular weight cut off (nMWCO) of the permselective membrane. Molecules larger than the nMWCO are physically impeded from traversing the membrane. Nominal molecular weight cut off is defined as 90% rejection under convective conditions. Typically the MWCO ranges between 50 and 200 kD, preferably between 50 and 100 kD.

Detailed Description Text (68):
In one embodiment, agarose hydrogels are used as a carried to present the laminin derived oligopeptide CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) to the site of nerve injury in an attempt to enhance nerve regeneration. Dorsal root ganglia have been shown to be responsive to CDPGYIGSR (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1) in vitro and show the greatest enhanced neuritic spread and neurite outgrowth compared to other fragments derived from laminin.

Detailed Description Text (73):
Characterization of Agarose Hydrogel Matrices

Detailed Description Text (83):
a. Hydraulic permeability: Gel blocks of different concentrations, each of thickness 0.5 cm and radius 1 cm, were mounted on a custom-built water column. Each block was subjected to a known hydraulic pressure, typically a 100 cm high H.sub.2 O column yielding approximately 24525 dynes/cm.sup.2. The hydraulic permeability per unit time for a given hydraulic pressure was measured for the various gel

concentrations. The average pore radius of the gel concentration range 0.5% to 5.0% was calculated as described by Refojo et al., J.Appl.Poly.Sci., 9, pp. 3417-26 (1965) using the hydraulic permeability.

Detailed Description Text (84):
The average pore radius, calculated from the hydraulic permeability measurements of the various agarose gels, decreased exponentially as the gel concentration increased (FIG. 1). E14 striatal cells did not extend neurities beyond a threshold agarose gel pore radius of 150 nm. The slope of the curve depicting pore radius was steep between gel concentrations of 1% and 2% indicating a strong dependence of pore size on gel concentration.

Detailed Description Text (85):
b. Scanning Electron microscopy (SEM): Agarose gels in the range 0.5% to 2.0% were freeze-dried, mounted on aluminum stubs, coated with gold and analyzed under a Joel 35M scanning electron microscope. Representative sections of the scanning electron micrographs were selected for evaluating the morphology and size of the pores.

Detailed Description Text (87):
c. Electron microscopy (ESEM): Agarose gels of the concentration range 0.5% to 2.5% were analyzed with an environmental scanning electron micrograph (Electroscan ESEM, type E3) under partially hydrated states to qualitatively asses gel pore morphology.

Detailed Description Text (88):
A decline in gel cavity radius was noted with increasing gel concentration. However, the nature and quality of images obtained with the ESEM allowed only qualitative conclusions on gel pore size to be drawn with c nfidence.

Detailed Description Text (89):
d. Gel electrophoresis: The electrophoretic mobility of insulin (Mw 5,700), bovine serum albumin (Mw. 66,000; radius 140 Angstroms) and bovine thyroglobulin (Mw. 669,000) in 1%, 2% and 4% agarose gels was measured under a constant electrophoretic voltage gradient. Twenty ml of the appropriate agarose gel concentration was poured into a DANAPHOR model 100 mini gel electrophoresis apparatus (Tectate S. S., Switzerland) with platinum electrodes. The proteins insulin, albumin and thyroglobulin were then subjected to a constant electrophoretic voltage gradient of 1 to 12V. The protein electrophoretic mobility was measured in the 1%, 2% and 4% agarose gels by measuring distance traveled per unit time. The relative electrophoretic velocity was then calculated after taking into account the isoelectric points of the different proteins, the v ltage employed and the time of exposure to enable electrophoretic mobility comparisons of insulin, albumin thyroglobulin in the agarose gels.

Detailed Description Text (102):
The peptides used were GRGDSP (GlyArgGlyAspSerPro; SEQ ID NO:2) (Telios pharmaceuticals, San Diego, Calif.), CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1), the 19-mer sequence CSRARKQAASIKVAVSADR (CysSerArg AlaArgLysGlnAlaAlaSerIleLysValAlaValSerAlaAspArg; SEQ ID NO:3), and x-IKVAV-x containing sequence (s-IleLysValAlaVal-x; AA.sub.11 -AA.sub.15 of SEQ ID NO:3) (Anawa, Wagen, Switzerland) and as a control, GGGGG (GlyGlyGlyGlyGly; SEQ ID NO:4) (Sigma). A cocktail of the three aforementioned peptides (PEPMIX) was also immobilized to the hydrogel backbone at a concentration of 2 mg each in a total of 5 ml buffer solution.

Detailed Description Text (108):
Gel porosity of underivatized agarose gels and glycine coupled agarose was determined as described in Example 1. The average pore radius of the gels were determined to be 310 nm for a 0.5% underivatized agarose gel and 360 nm for a 0.5% glycine coupled agarose gel using the water column for hydraulic permeability

measurements.

Detailed Description Text (112):
Agarose hydrogels supported neurite outgrowth from DRGs in both X-Y and X-Z planes, demonstrating the 3-D character of neurite outgrowth in agarose gels. Fluorescein diacetate assay showed viable DRG neurons after 6 days in culture in all agarose gels used.

Detailed Description Text (122):
The effect of derivatized agarose gels on the regeneration of transected rat spinal dorsal roots was evaluated by using 6 mm long polymer guidance channels filled with CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1)--agarose to bridge a 4 mm gap in a transected dorsal root model. After 4 weeks, significantly greater numbers of myelinated axons were observed in the channels filled with CDPGYIGSR (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1)--agarose gels compared to channels filled with underivatized agarose gels.

Detailed Description Text (133):
Semi-thin cross-sections along the length of the guidance channel showed that myelinated axons were present all along the 4 mm nerve gap. Histological sections of guidance channels filled with agarose gels carrying regenerated dorsal roots showed doughnut shaped, centrally located nerve cables. Tissue reaction to the PAN/PVC polymer consisted of multi-nucleate giant cell and connective tissue infiltration. Neovascularisation was evident in the midst of regenerated nervous tissue along the Schwann cell infiltration. Light micrographs of regenerating dorsal root cables at 4 weeks post-implantation at the mid-point of guidance channels showed comparatively more nervous tissue in channels containing CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) gels relative to channels with underivatized agarose. At the mide-point of the 4 mm nerve gap, the channel filled with AgCDPGYIGSR (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1) had a significantly greater (p-0.05) number of myelinated axons compared to the channels with agarose plain gels (see FIG. 7). The number of myelinated axons at the midpoint in the agarose plain filled channels were comparable to those in saline filled channels described by McCormack et al., J.Comp. Neurol., 313, pp. 449-56 (1991). CDPGYIGSR-derivatized (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1) agarose gels had a significantly higher density ($p<0.05$) of myelinated axons at 0.5 mm and 2.0 mm along the channel at 4 weeks postimplantation (FIG. 8). The density of myelinated axons is defined as the number of myelinated axons per 10.sup.5 square microns of cable area.

Detailed Description Text (135):
All of the 10 mm long guidance channels implanted to bridge transected sural nerves were kinked due to flexion at the rats' knee-joint. Histological evaluation of nerves proximal to the kink showed regenerated cables located at the center of the channel with myelinated axons. All cables present in the channel distal to the kink contained a fibrotic cable but no myelinated axons. Almost all of the kinks occurred between 2-4 mm into the nerve gap. Therefore only the 2 mm point was analyzed for myelinated axons in this study. Light micrographs of regenerating sural nerves, 4 weeks post-implantation at the 2.0 mm point in polymer guidance channels filled Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) showed nrelatively higher numbers of nerve fascicles and eovascularization than in the other guidance channels. At 2 mm into the 8 mm nerve gap, guidance channels filled with AgCDPGYIGSR (CysAspPro GlyTyrIleGlySerArg; SEQ ID NO:1) gels had a significantly greater number ($p<0.05$) of myelinated axons compared to either the saline filled channels or agarose plain filled channels. See FIG. 9. At this point, the density of myelinated axons in the CDPGYIGSR-derivatized (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) agarose channels was also significantly greater ($p<0.05$) than the density of axons in the agarose-plain and saline-filled channels (FIG. 10). When the number of myelinated axons was compared to the number present in a normal control sural nerve, at 4 weeks, the average number of

myelinated axons regenerated across the saline filled channel was 12% of control sural nerve, 13% of control nerve for AgPlain filled channels and 40% of control sural nerve for AgCDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) filled channels.

Detailed Description Text (141):
This data indicates the feasibility of developing a matrix designed to enhance nerve regeneration by coupling neurite promoting biomolecules to agarose hydrogels.

Other Reference Publication (7):
Aebischer, P., et al., "Macroencapsulation of Dopamine-Secreting Cells By Coextrusion With an Organic Polymer Solution," Biomaterials, 12, pp. 50-56 (1991).

Other Reference Publication (21):
[illegible]

With The Growth Substrate," Neuroscience, 59, pp. 43-54 (1994).

Other Reference Publication (34):
Friedlander, David R., et al., "Functional Mapping of Cytotactin: Proteolytic Fragments Active in Cell-Substrate Adhesion," J. Cell Biol., 107, pp. 2329-2340 (1988).

Other Reference Publication (62):
Massia, Stephen P., and Jeffrey A. Hubbell, "Covalent Surface Immobilization of Arg-Gly-Asp- and Try-IIe-Gly-Ser-Arg-Containing Peptides to Obtain Well-Defined Cell-Adhesive Substrates," Analytical Biochemistry, 187, pp. 292-301 (1990).

Other Reference Publication (77):
Refojo, Miguel F., "Permeation of Water Through Some Hydrogels," J. Applied Polymer Science, 9, pp. 3417-3426 (1965).

Other Reference Publication (85):
Smalheiser, Neil R., et al., "Laminin As Substrate for Retinal Axons In Vitro," Dev. Brain Research, 12, pp. 136-140 (1984).

Other Reference Publication (94):
Woerly, S., et al., "Synthetic Polymer Matrices for Neural Cell Transplantation," Cell Transplantation, 2, pp. 229-239 (1993).

CLAIMS:

1. A bioartificial extracellular matrix comprising a three-dimensional high water content derivatized hydrogel matrix having a hydrogel matrix core

(a) wherein the hydrogel matrix is derivatized through the matrix by covalent-immobilization of at least one cell adhesive peptide fragment, homogeneously dispersed throughout the hydrogel matrix, and

(b) wherein the hydrogel matrix has an average pore radius greater than 120 nm.

5. The matrix according to any one of claims 1 and 2-4, wherein the hydrogel matrix is a polysaccharide hydrogel matrix.

6. The matrix according to claim 1, wherein the hydrogel matrix is an agarose hydrogel matrix.

7. The matrix according to claim 6, wherein the agarose concentration in the hydrogel matrix ranges between 0.5-1.25% (w/v) and the hydrogel matrix has an

average pore radius ranging between 120-290 nm.

8. The matrix according to claim 6, wherein the agarose concentration in the hydrogel matrix is 1.0% (w/v) and the hydrogel matrix has an average pore radius of approximately 150 nm.

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DOCUMENT-IDENTIFIER: US 6156572 A
TITLE: Bioartificial extracellular matrix containing hydrogel matrix derivatized with cell adhesive peptide fragment

Abstract Text (1):
A bioartificial extracellular matrix for use in tissue regeneration or replacement is provided by derivatizing a three-dimensional hydrogel matrix with a cell adhesive extracellular matrix protein or cell adhesive peptide fragment of the protein. Preferably, derivatizing is by covalent immobilization of a cell adhesive peptide fragment having the amino acid sequence, ArgGlyAsp, TyrIleGlySerArg or IleLysValAlaVal. Cartilage or tendon can be regenerated by implanting a matrix containing an adhesive peptide fragment that favors chondrocyte invasion. The matrix can be pre-seeded with cells, and tissue can be reconstituted in vitro and then implanted. A cell-seeded matrix can be encapsulated in a semi-permeable membrane to form a bioartificial organ. An agarose hydrogel matrix having an agarose concentration of 0.5-1.25% (w/v) and an average pore radius between 120 nm and 290 nm is preferred. A nerve guidance channel for use in regenerating severed nerve is prepared containing a tubular semi-permeable membrane having openings adapted to receive ends of a severed nerve, and an inner lumen containing the hydrogel matrix having a bound cell adhesive peptide fragment through which the nerve can regenerate.

Brief Summary Text (13):
All of the studies using these preptidic sequences of cell attachment and neurite promotion were conducted on flat two-dimension substrates (Smallheiser et al., Dev. Brain Res., 12, pp. 136-40 (1984); Graf et al., Biochemistry, 26, pp. 6896-900 (1987); Sephel et al., Biochem. Biophys. Res. Comm., 2, pp. 821-29 (1989); Jucker et al., J. Neurosci. Res., 28, pp. 507-17 (1991)). The physical and chemical nature of the culture substrate influences cell attachment and neurite extension. The physical microstructure of a 2-D culture substrate can influence cell behavior. The use of permissive and on-permissive culture surface chemistries facilitates nerve guidance in 2-D. The cell attachment regulating function of various serum proteins like albumin and fibronectin is dependent on the chemistries of the culture substrates that they are adsorbed onto.

Brief Summary Text (14):
Gene expression is reported to be regulated differently by a flat 2-D substrate as opposed to a hydrated 3-D substrate. For example, monolayer culture of primary rabbit articular chondrocyte and human epiphyseal chondrocyte on 2-D tissue culture substrates causes primary chondrocyte to lose their differentiated phenotype. The differentiated chondrocyte phenotype is re-expressed when they are cultured in 3-D agarose gels (Benya and Shaffer, Cell, 30, pp. 215-24 (1982); Aulthouse, et al., In Vitro Cell Dev. Bio., 25, pp. 659-68 (1989)).

Brief Summary Text (19):
This invention provides a three-dimensional hydrogel based, biosynthetic, extracellular matrix (ECM) equivalent, and method of making same. Agarose matrices having a chemistry amenable to derivatization with various ECM adhesive peptides and proteins, are preferred in forming the 3-D hydrogel substrates of this invention. These biologically active 3-D templates may be useful in facilitating

tissue regeneration or replacement.

Drawing Description Text (2):
FIG. 1. A double Y-axis plot depicting the influence of agarose gel concentration on average pore radius (Y1) and percent striatal cells extending neurites (Y2) after 72 hours in culture. Pore radius was calculated by hydraulic permeability measurements of the different gel concentrations. Solid line through pore radii data points is an exponential fit with r.sup.2 =0.985.

Drawing Description Text (8):
FIG. 7. Graph showing the number of myelinated axons regenerated at 4 weeks along polymer guidance channels filled with AgPlain and Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) gels. "*" depicts a statistically significant difference with p<0.05 using the Student t test.

Drawing Description Text (9):
FIG. 8. Graph showing the density of myelinated axons regenerated at 4 weeks along polymer guidance channels filled with AgPlain and Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) gels. "*" depicts a statistically significant difference with p<0.05 using the Student t test.

Drawing Description Text (10):
FIG. 9. Histogram depicting the number of myelinated axons in regenerating sural nerves at 2.0 mm distance from the proximal nerve stump in polymer guidance channels filled with A) saline; B) A Plain and C) Ag-CDPGYIGSR g (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1). "*" depicts a statistical difference of p<0.05 when compared to saline or AgPlain. Student t test was used to evaluate statistical significance.

Drawing Description Text (11):
FIG. 10. Histogram depicting the density of myelinated axons in regenerating sural nerves at 2.0 mm distance from the proximal nerve stump in polymer guidance channels filled with A) saline; B) AgPlain and C) Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1). "*" depicts a statistical difference of p<0.05 when compared to saline or AgPlain. Student t test was used to evaluate statistical significance.

Detailed Description Text (2):
This invention provides a biosynthetic, hydrogel-based, three-dimensional bioartificial ECM. The bioartificial extracell lar matricesuof this invention offer the possibility of manipulating cells in 3-D, and may be used as three dimensional templates for tissue engineering efforts in vitro and in vivo.

Detailed Description Text (5):
Any suitable hydrogel may be used as the substrate for the bioartificial extracellular matrices of this invention. Compositions that form hydrogels fall into three classes. The first class carries a net negative charge (e.g., alginate). The second class carries a net positive charge (e.g., collagen and laminin). Examples of commercially available extracellular matrix component hydrogels include Matrigel.TM. and Vitrogen.TM.. The third class is net neutral in charge (e.g., highly crosslinked polyethylene oxide, or polyvinylalcohol).

Detailed Description Text (6):
A hydrogel suitable for use in this invention is preferably a defined polymer, most preferably a polymer that is synthetic or can be prepared from a naturally occurring, non-tumorigenic source, free of undesired biological (e.g., bacterial or viral), chemical or other contaminants. Most preferred as the matrix substrate are well characterized hydrogels that permit presentation of only the desired ECM adhesion molecule or adhesive peptide fragement in 3-D, substantially free of undesired adhesion motifs.

Detailed Description Text (7):
Matrigel.TM. is not a defined substrate and also less desirable since it is derived from a murine sarcoma line. In addition, not all synthetic polymer hydrogels are suitable. For example, the use of acrylic based hydrogels by Woerly et al., Cell Transplantation, 2, pp. 229--39 (1993) presents the possibility of cytotoxicity because entrapment of neuronal cells is done concomitantly with the cross-linking reaction in the presence of free radical initiators.

Detailed Description Text (8):
Polymers that may be useful hydrogel matrix substrate materials include high molecular weight polyethylene oxide (PEO) and hyaluronate. Stabilized hyaluronate is commercially available (Fidia Advanced Biopolymers). Various PEO polymers are also commercially available.

Detailed Description Text (9):
Polysaccharides are a class of macromolecules of the general formula (CH.sub.2 O).sub.n which are useful as the hydrogel substrate in the present invention. Polysaccharides include several naturally occuring compounds, e.g., agarose, alginate and chitosan. We prefer agarose.

Detailed Description Text (10):
Agarose is a clear, thermoreversible hydrogel made of polysaccharides, mainly the alternating copolymers of 1,4 linked and 3,6-anhydro-.alpha.-L-galactose and 1,3 linked .beta.-D-galactose. Two commercially available agaroses are SeaPrep.RTM. and SeaPlaque.RTM. agarose (FMC Corp. Rockland, Me.). SeaPrep.RTM. is a hydro yethylated agarose that gels at 17.degree. C. The particular suitability of a hydrogel as a biomaterial stems from the similarity of its physical properties to those of living tissues. This resemblance is based on its high water content, soft rubbery consistency and low interfacial tension. The thermoreversible properties of agarose gels make it possible for agarose to be a liquid at room temperature allowing for easy mixing of cell-gel solution and then cooling to 4.degree. C. causes gelation and entrapment of cells. This is a comparatively benign process, free of chemicals toxic to the cells.

Detailed Description Text (11):
We prefer an agarose concentration of 0.50 to 1.25% (w/v), most preferably 1.0%, for the permissive layers of the hydrogel matrix.

Detailed Description Text (12):
Several physical properties of th hydrogel matrices of this invention are dependent upon gel concentration. Increase in gel concentration may change the gel pore radius, morphology, or its permeability to different molecular weight proteins.

Detailed Description Text (13):
Gel pore radius determination can be determined by any suitable method, including hydraulic permeability determination using a graduated water column, transmission electron microscopy and sieving spheres of known radius through different agar gel concentrations. See, e.g., Griess et al., Biophysical J., 65, pp. 138-48 (1993). We prefer hydraulic permeability-based pore radius determination, as the method most sensitive to changes in gel concentration.

Detailed Description Text (14):
Measurement of gel hydraulic permeability using algraduated water column enab ed the calculation of average pore radius for each of the gel concentrations studied. The average gel pore radius fall exponentially as the gel concentration increased. The slope of the curve indicated the sensitivity of pore radius to gel concentration. The average gel pore radius preferably varies between 120-290 nm, and is most preferably approximately 150 nm. The pore radius of the 1.25% threshold

agarose gel concentration was 150 nm.

Detailed Description Text (15):
The agarose hydrogels of this invention may be used as a carrier to present various ECM proteins or peptides, e.g., laminin fibronectin, and/or their peptidic analogs in 3-D. The chemistry of agarose permits easy modification with such ECM adhesive proteins and/or peptides. We prefer covalent immobilization of ECM proteins to the hydrogel backbone. Such immobilization is important because the physical blending of low molecular weight oligopeptides with hydrogels will not retain the peptides in the gel. Further, covalent immobilization prevents the possible saturation of cell surface receptors by `free-floating` ECM molecules in hydrogel-ECM molecule blends.

Detailed Description Text (17):
The bioartificial hydrogel extracellular matrices of this invention are useful for presenting in 3-D full length extracellular matrix proteins involved in cell adhesion. In addition, peptide fragments of such adhesion molecules that contain cell binding sequences may also be used (i.e., adhesive peptide fragement). Several such adhesive peptide fragments are known in the art. A particular peptide fragment can be tested for its binding ability or adhesive capacity according to standard techniques.

Detailed Description Text (18):
The bioartificial hydrogel matrices of this invention can be used to present ECM adhesion molecules, or adhesive peptide fragments thereof, in 3-D to a variety of cell types. These cell types include any cell that is normally in contact with the ECM in vivo, or any cell bearing a cell surface receptor capable of binding to an ECM adhesion molecule or adhesive peptide fragment thereof.

Detailed Description Text (24):
In some embodiments, the hydrogel ECM matrix can be derivatized with the appropriate ECM adhesion molecules or adhesive peptide fragments and implanted into a desired location in a host, e.g., a mammal, preferably a human. In these embodiments, the matrix acts as a support for tissue regeneration, whereby the host cells infiltrate the matrix. In the presence of the appropriate 3-D molecular cues in the matrix host tissue regeneration is facilitated.

Detailed Description Text (26):
In other embodiments, the bioartificial matrices of this invention can be pre-seeded with cells, whereby the cells are suspended in the matrix and exposed to the appropriate molecular cues in 3-D. These cell-seeded ma rices are useful in tissue replacement protocols. According to these embodiments, tissue can be reconstituted in vitro and them implanted into a host in need thereof. For example, cardiac myoblasts may be suspended in the derivatized hydrogel matrices of this invention to create a tissue patch of a thickness corresponding to the cardiac wall. The reconstituted cardiac patch could then be implanted, as part of a tissue replacement therapy.

Detailed Description Text (27):
Similar protocols for cartilage, tendon, bone, skin, nerve, blood vessels and other tissues are contemplated. The ability to cast hydrogels, e.g., agarose, into a variety of shapes, as well the ability to fabricate "permissive" gel concentrations enables the production of bioartificial matrices that can influence cell behavior in defined planes or through defined "tracts".

Detailed Description Text (31):
CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) has been shown to evoke only 30% of the maximal response obtained by laminin in chemotactic functions with melanoma cells (Graf. et al., Biochemistry, 26, pp. 6896-900 (1987)). Thus, the use of full length ECM molecules may elicit more significant cellular effects. However,

the use of minimal oligopeptides creates a more stringent substrate condition and facilitates the testing of the gel system without the potent biological effects of full length proteins eclipsing the gel's physical effects. This enables the development and testing of a system with a base physical structure to support cell viability and influence cell behavior. The hydrogel matrix can then be rendered progressively more permissive by the use of appropriate covalently-coupled cell adhesion or extracellular matrix molecules.

Detailed Description Text (33):
In another preferred embodiment, the compositions of this invention may be used in neural cell transplantation. The ability of biosynthetic hydrogels to organize, support and direct neurite extension from neural cells may also be useful for applications such as 3-D neural cell culture and nerve regeneration. The bioartificial extracellular matrices of this invention may potentially carry one or more of the several cell adhesion molecules that have been identified to play an important role in cell migration and neurite extension in the developing nervous system, including N-CAM and Ng-CAM (Crossin et al., Proc. Natl. Acad. Sci., 82, pp. 6942-46 (1985); Daniloff et al., J. Neurosci., 6, pp. 739-58 (1986)), tenascin (Wehrle et al., Development, 1990, pp. 401-15 (1990) and L1 (Nieke and Schachner, Differentiation, 30, pp. 141-51 (1985)). Among extracellular matrix glycoproteins, laminin has been shown to be one of the most potent inducers of neurite outgrowth in vitro. It is a component of the Schwann cell basal lamina and is thought to be involved in axonal regeneration in vivo (Baron-Van-Evercooren et al., J. Neurosci. Res., 8, pp. 179-83 (1983); Manthorpe et al., J. Cell. Biol., 97, pp. 1882-90 (1983); Rogers et al., Dev. Biol., 113, pp. 429-35 (1983).

Detailed Description Text (51):
The agarose hydrogel compositions of this invention may be useful in nerve guidance channels. Such nerve guidance channels are well known in the art. Synthetic guidance channels have been used as inert conduits providing axonal guidance, maintaining growth factors, and preventing scar tissue invasion. Permselective channels with a molecular weight cut-off of 50,000 daltons allowed regeneration of nerves in a mouse sciatic nerve model. The regenerated nerves were characterized by fine epineurium and high numbers of myelinated axons. Aebischer et al., "The Use Of A Semi-Permeable Tube As A Guidance Channel For A Transected Rabbit Optic Nerve", In Gash & Sladek [Eds] Progress in Brain Research, 78, pp. 599-603 (1988).

Detailed Description Text (54):
The nerve guidance channels of the present invention include an implantable, biocompatible tubular permselective membrane having openings to receive the severed nerve. The lumen of the membrane preferably has a diameter ranging from about 0.5 mm to about 2.0 cm, to permit the nerve to regenerate through it. The thickness of the membrane may range from about 0.05 to about 1.0 mm. In some embodiments the membrane has a molecular weight cut-off of about 100,000 daltons or less. The membrane is preferably impermeable to fibroblasts and other scar-forming connective tissue cells. Additionally, the membrane may be composed of a biodegradable material. An agarose matrix is disposed in the lumen of the nerve guidance channel. The agarose concentration should range between 0.5 to 1.25%, preferably 1.0%. The average gel pore radius can vary between 120 to 290 nm, and is most preferably approximately 150 nm.

Detailed Description Text (55):
The optimal concentration of agarose gel for use as a regeneration matrix will vary according to the intended use of the matrix. The optimal concentration for in vitro use may not be optimal for the in vivo milieu. Neurite outgrowth in agarose gels is strongly dependent upon the pore size of agarose gels. Syneresis at the channel mid-point could alter the pore size of agarose gels enough to inhibit regeneration and therefore result in the absence of nerve cable in the mid-portion of the regeneration nerve bundle. It is important to account and if possible, correct for syneresis of the gel at channel mid-point. This may be overcome by two strategies.

One, the use of more dilute agarose gels to fill the channels may accommodate syneresis in the middle and still retain the pore size of gel at the channel midpoint to ranges permissible for neurite extension. Second, the use of a rough inner membrane of the channel may serve to prevent the fibroblasts induced syneresis of the gel inside the guidance channel (Aebischer et al., Brain Research, 531, pp. 21-18 (1990)).

Detailed Description Text (60):
The nerve guidance channels of this invention may additionally be seeded with Schwann cells. Schwann cells resident in the peripheral nerve trunk play a crucial role in the regenerative process. Schwann cells seeded in permselective synthetic guidance channels support extensive peripheral nerve regeneration. Schwann cells secrete laminin, which possesses neurite-promoting activity in vitro. See, e.g., Aebischer et al., Brain Research, 454, pp. 179-87 (1988). The Schwann cells are preferably longitudinally oriented along the guidance channel. This can be achieved by thermal manipulation of the agarose gel to orient the pores longitudinally, using methods well known in the art.

Detailed Description Text (63):
Briefly, various polymers and polymer blends can be used to manufacture the nerve guidance channel. Polymeric membranes, forming the nerve guidance channel may include polyacrylates (including acrylic copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethane, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones, polyphosphazenes, polyacrylonitriles, poly (acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof.

Detailed Description Text (65):
The jacket may have a single skin (Type 1, 2), or a double skin (Type 4). A single-skinned hollow fiber may be produced by quenching only one of the surfaces of the polymer solution as it is co-extruded. A double-skinned hollow fiber may be produced by quenching both surfaces o the polymer solution as it is co-extruded. Typically, a greater percentage of the outer surface of Type 1 hollow fibers is occupied by macropores compared to Type 4 hollow fibers. Type 2 hollow fibers are intermediate.

Detailed Description Text (66):
The jacket of the nerve guidance channel will have a pore size that determines the nominal molecular weight cut off (nMWCO) of the permselective membrane. Molecules larger than the nMWCO are physically impeded from traversing the membrane. Nominal molecular weight cut off is defined as 90% rejection under convective conditions. Typically the MWCO ranges between 50 and 200 kD, preferably between 50 and 100 kD.

Detailed Description Text (68):
In one embodiment, agarose hydrogels are used as a carried to present the laminin derived oligopeptide CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) to the site of nerve injury in an attempt to enhance nerve regeneration. Dorsal root ganglia have been shown to be responsive to CDPGYIGSR (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1) in vitro and show the greatest enhanced neuritic spread and neurite outgrowth compared to other fragments derived from laminin.

Detailed Description Text (73):
Characterization of Agarose Hydrogel Matrices

Detailed Description Text (83):
a. Hydraulic permeability: Gel blocks of different concentrations, each of thickness 0.5 cm and radius 1 cm, were mounted on a custom-built water column. Each block was subjected to a known hydraulic pressure, typically a 100 cm high H.sub.2 O column yielding approximately 24525 dynes/cm.sup.2. The hydraulic permeability per unit time for a given hydraulic pressure was measured for the various gel

concentrations. The average pore radius of the gel concentration range 0.5% to 5.0% was calculated as described by Refojo et al., J.Appl.Poly.Sci., 9, pp. 3417-26 (1965) using the hydraulic permeability.

Detailed Description Text (84):
The average pore radius, calculated from the hydraulic permeability measurements of the various agarose gels, decreased exponentially as the gel concentration increased (FIG. 1). E14 striatal cells did not extend neurities beyond a threshold agarose gel pore radius of 150 nm. The slope of the curve depicting pore radius was steep between gel concentrations of 1% and 2% indicating a strong dependence of pore size on gel concentration.

Detailed Description Text (85):
b. Scanning Electron microscopy (SEM): Agarose gels in the range 0.5% to 2.0% were freeze-dried, mounted on aluminum stubs, coated with gold and analyzed under a Joel 35M scanning electron microscope. Representative sections of the scanning electron micrographs were selected for evaluating the morphology and size of the pores.

Detailed Description Text (87):
c. Electron microscopy (ESEM): Agarose gels of the concentration range 0.5% to 2.5% were analyzed with an environmental scanning electron micrograph (Electroscan ESEM, type E3) under partially hydrated states to qualitatively asses gel pore morphology.

Detailed Description Text (88):
A decline in gel cavity radius was noted with increasing gel concentration. However, the nature and quality of images obtained with the ESEM allowed only qualitative conclusions on gel pore size to be drawn with confidence.

Detailed Description Text (89):
d. Gel electrophoresis: The electrophoretic mobility of insulin (Mw 5,700), bovine serum albumin (Mw. 66,000; radius 140 Angstroms) and bovine thyroglobulin (Mw. 669,000) in 1%, 2% and 4% agarose gels was measured under a constant electrophoretic voltage gradient. Twenty ml of the appropriate agarose gel concentration was poured into a DANAPHOR model 100 mini gel electrophoresis apparatus (Tectate S. S., Switzerland) with platinum electrodes. The proteins insulin, albumin and thyroglobulin were then subjected to a constant electrophoretic voltage gradient of 1 to 12V. The protein electrophoretic mobility was measured in the 1%, 2% and 4% agarose gels by measuring distance traveled per unit time. The relative electrophoretic velocity was then calculated after taking into account the isoelectric points of the different proteins, the voltage employed and the time of exposure to enable electrophoretic mobility comparisons of insulin, albumin thyroglobulin in the agarose gels.

Detailed Description Text (102):
The peptides used were GRGDSP (GlyArgGlyAspSerPro; SEQ ID NO:2) (Telios pharmaceuticals, San Diego, Calif.), CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1), the 19-mer sequence CSRARKQAASIKVAVSADR (CysSerArg AlaArgLysGlnAlaAlaSerIleLysValAlaValSerAlaAspArg; SEQ ID NO:3), and x-IKVAV-x containing sequence (s-IleLysValAlaVal-x; AA.sub.11 -AA.sub.15 of SEQ ID NO:3) (Anawa, Wagen, Switzerland) and as a control, GGGGG (GlyGlyGlyGlyGly; SEQ ID NO:4) (Sigma). A cocktail of the three aforementioned peptides (PEPMIX) was also immobilized to the hydrogel backbone at a concentration of 2 mg each in a total of 5 ml buffer solution.

Detailed Description Text (108):
Gel porosity of underivatized agarose gels and glycine coupled agarose was determined as described in Example 1. The average pore radius of the gels were determined to be 310 nm for a 0.5% underivatized agarose gel and 360 nm for a 0.5% glycine coupled agarose gel using the water column for hydraulic permeability

measurements.

Detailed Description Text (112):
Agarose hydrogels supported neurite outgrowth from DRGs in both X-Y and X-Z planes, demonstrating the 3-D character of neurite outgrowth in agarose gels. Fluorescein diacetate assay showed viable DRG neurons after 6 days in culture in all agarose gels used.

Detailed Description Text (122):
The effect of derivatized agarose gels on the regeneration of transected rat spinal dorsal roots was evaluated by using 6 mm long polymer guidance channels filled with CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1)--agarose to bridge a 4 mm gap in a transected dorsal root model. After 4 weeks, significantly greater numbers of myelinated axons were observed in the channels filled with CDPGYIGSR (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1)--agarose gels compared to channels filled with underivatized agarose gels.

Detailed Description Text (133):
Semi-thin cross-sections along the length of the guidance channel showed that myelinated axons were present all along the 4 mm nerve gap. Histological sections of guidance channels filled with agarose gels carrying regenerated dorsal roots showed doughnut shaped, centrally located nerve cables. Tissue reaction to the PAN/PVC polymer consisted of multi-nucleate giant cell and connective tissue infiltration. Neovascularisation was evident in the midst of regenerated nervous tissue along the Schwann cell infiltration. Light micrographs of regenerating dorsal root cables at 4 weeks post-implantation at the mid-point of guidance channels showed comparatively more nervous tissue in channels containing CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) gels relative to channels with underivatized agarose. At the mide-point of the 4 mm nerve gap, the channel filled with AgCDPGYIGSR (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1) had a significantly greater (p-0.05) number of myelinated axons compared to the channels with agarose plain gels (see FIG. 7). The number of myelinated axons at the midpoint in the agarose plain filled channels were comparable to those in saline filled channels described by McCormack et al., J.Comp. Neurol., 313, pp. 449-56 (1991). CDPGYIGSR-derivatized (CysAspProGly TyrIleGlySerArg; SEQ ID NO:1) agarose gels had a significantly higher density ($p<0.05$) of myelinated axons at 0.5 mm and 2.0 mm along the channel at 4 weeks postimplantation (FIG. 8). The density of myelinated axons is defined as the number of myelinated axons per 10.sup.5 square microns of cable area.

Detailed Description Text (135):
All of the 10 mm long guidance channels implanted to bridge transected sural nerves were kinked due to flexion at the rats' knee-joint. Histological evaluation of nerves proximal to the kink showed regenerated cables located at the center of the channel with myelinated axons. All cables present in the channel distal to the kink contained a fibrotic cable but no myelinated axons. Almost all of the kinks occurred between 2-4 mm into the nerve gap. Therefore only the 2 mm point was analyzed for myelinated axons in this study. Light micrographs of regenerating sural nerves, 4 weeks post-implantation at the 2.0 mm point in polymer guidance channels filled Ag-CDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) showed relatively higher numbers of nerve fascicles and neovascularization than in the other guidance channels. At 2 mm into the 8 mm nerve gap, guidance channels filled with AgCDPGYIGSR (CysAspPro GlyTyrIleGlySerArg; SEQ ID NO:1) gels had a significantly greater number ($p<0.05$) of myelinated axons compared to either the saline filled channels or agarose plain filled channels. See FIG. 9. At this point, the density of myelinated axons in the CDPGYIGSR-derivatized (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) agarose channels was also significantly greater ($p<0.05$) than the density of axons in the agarose-plain and saline-filled channels (FIG. 10). When the number of myelinated axons was compared to the number present in a normal control sural nerve, at 4 weeks, the average number of

myelinated axons regenerated across the saline filled channel was 12% of control sural nerve, 13% of control nerve for AgPlain filled channels and 40% of control sural nerve for AgCDPGYIGSR (CysAspProGlyTyrIleGlySerArg; SEQ ID NO:1) filled channels.

Detailed Description Text (141):
This data indicates the feasibility of developing a matrix designed to enhance nerve regeneration by coupling neurite promoting biomolecules to agarose hydrogels.

Other Reference Publication (7):
Aebischer, P., et al., "Macroencapsulation of Dopamine-Secreting Cells By Coextrusion With an Organic Polymer Solution," Biomaterials, 12, pp. 50-56 (1991).

Other Reference Publication (21):
Chu, C.H., and A.M. Tolkovsky, "Alternative Adrenal Chromaffin Cell Fates Induced by Basic Fibroblast Growth Factor or Cyclic AMP In Vitro Depend on a Collaboration With The Growth Substrate," Neuroscience, 59, pp. 43-54 (1994).

Other Reference Publication (34):
Friedlander, David R., et al., "Functional Mapping of Cytotactin: Proteolytic Fragments Active in Cell-Substrate Adhesion," J. Cell Biol., 107, pp. 2329-2340 (1988).

Other Reference Publication (62):
Massia, Stephen P., and Jeffrey A. Hubbell, "Covalent Surface Immobilization of Arg-Gly-Asp- and Try-IIe-Gly-Ser-Arg-Containing Peptides to Obtain Well-Defined Cell-Adhesive Substrates," Analytical Biochemistry, 187, pp. 292-301 (1990).

Other Reference Publication (77):
Refojo, Miguel F., "Permeation of Water Through Some Hydrogels," J. Applied Polymer Science, 9, pp. 3417-3426 (1965).

Other Reference Publication (85):
Smalheiser, Neil R., et al., "Laminin As Substrate for Retinal Axons In Vitro," Dev. Brain Research, 12, pp. 136-140 (1984).

Other Reference Publication (94):
Woerly, S., et al., "Synthetic Polymer Matrices for Neural Cell Transplantation," Cell Transplantation, 2, pp. 229-239 (1993).

CLAIMS:

1. A bioartificial extracellular matrix comprising a three-dimensional high water content derivatized hydrogel matrix having a hydrogel matrix core

(a) wherein the hydrogel matrix is derivatized through the matrix by covalent-immobilization of at least one cell adhesive peptide fragment, homogeneously dispersed throughout the hydrogel matrix, and

(b) wherein the hydrogel matrix has an average pore radius greater than 120 nm.

5. The matrix according to any one of claims 1 and 2-4, wherein the hydrogel matrix is a polysaccharide hydrogel matrix.

6. The matrix according to claim 1, wherein the hydrogel matrix is an agarose hydrogel matrix.

7. The matrix according to claim 6, wherein the agarose concentration in the hydrogel matrix ranges between 0.5-1.25% (w/v) and the hydrogel matrix has an

average pore radius ranging between 120-290 nm.

8. The matrix according to claim 6, wherein the agarose concentration in the hydrogel matrix is 1.0% (w/v) and the hydrogel matrix has an average pore radius of approximately 150 nm.

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DOCUMENT-IDENTIFIER: US 6013391 A
TITLE: High capacity high rate materials

Detailed Description Text (2):
V.sub.2 O.sub.5 0.5H.sub.2 O xerogels (XRG), cryogels (CRG) and aerogels (ARG) provide excellent intercalation host materials for lithium ions (Li.sup.+) when synthesized according to this invention. The increased intercalation capacity makes these materials useful for battery electrodes, electrochromic displays and other electrochemical devices. The materials described hereinbelow show the highest capacity and highest energy density of any vanadium oxide. They are also more energetic than heretofore reported values for lithiated oxides of manganese, cobalt and nickel. The materials described herein exhibit the highest columbic capacity of any of the intercalation hosts yet discovered.

Detailed Description Text (4):
Precursor Hydrogel V.sub.2 O.sub.5.nH.sub.2 O

Detailed Description Text (5):
The initial step in practicing the invention involves preparing a precursor hydrogel of the V.sub.2 O.sub.5. This may be effected in a variety of ways known in the art but a preferred procedure is to synthesize the hydrogel from a solution of metavanadate salt such as for example the sodium form which is passed through an ion exchange column in which the sodium ions are exchanged with hydrogen ions to produce decavanadic acid which is collected in fractions. The middle fractions (.about.50%) are allowed to set without disturbance. Self-assembly of V.sub.2 O.sub.5 fibrils and ribbons occurs and in a first stage a colloidal "sol" is formed. Further maturation produces a uniform viscous "gel" in a second stage. The gel network consists of interconnected solid and water phases. The concentration and temperature influence the rate of maturation to the gel state and also its viscosity. The time can change from a few minutes at high concentrations to weeks or months at low concentrations. A preferred concentration range of the metavanadate salt is 0.1 moles/liter to 0.5 moles/liter. The time for aging to maturation in this case ranges from one week to two months. Increased concentration of decavanadic acid precursor, increased time, increased temperature and increased (but relatively low) concentrations of V(IV), all increase the rate of self-assembly of the ribbons.

Detailed Description Text (7):
Having obtained the gel network (precursor hydrogel V.sub.2 O.sub.5.nH.sub.2 O) it may then be used with further processing as a source of the materials of the invention. Generally, the further processing involves the removal of additional water while preserving the network, resulting in microporous solid material V.sub.2 O.sub.4.5 (OH) with high surface area, a red color of the material is preferred.

Detailed Description Text (8):
The pores which are filled with water in the gel have different sizes for different hydration states of the gel. Thus, by changing the concentration of the liquid gel, one can deliberately manipulate the pore size to obtain product materials of predetermined surface area, catalytic activity and kinetic capability. For example, 0.5M sodium metavanadate gives V.sub.2 O.sub.5.200H.sub.2 O gel and 0.1M gives

V.sub.2 O.sub.5.1000H.sub.2 O.

Detailed Description Text (15):
A thin film material may be prepared by spin coating the precursor hydrogel. This is described in Example 2.

Detailed Description Text (17):
Precursor hydrogel was spin coated onto metal (Ni or Au) coated quartz and silicon substrates. About 0.2 cubic centimeter of hydrogel per square centimeter of substrate was placed on the spin coater at 2000 rpm and after 60 seconds a thin coat of XRG was formed. This was further dried by slow evacuation at 25.degree. C. for 16 hours to the nominal experimental stoichiometry V.sub.2 O.sub.4.5 (OH) and nominal surface area of 10 m .sup.2 /g. A 30 .mu.g/cm.sup.2 film 0.1 .mu.m thick was formed on the substrate piece of it was then used as an electrode for test purposes. The electrode was placed in a solution of 1M Li ClO.sub.4 in propylene carbonate and cycled against lithium. The test cell was assembled in a dry box. The resulting FIG. 2 shows that 4 Li.sup.+ ions are inserted reversibly as the electrode cycles between 3.5 and 1.5V. This corresponds to a specific capacity greater than 2000 coulombs/gram and a specific energy of greater than 1000 Wh/Kg for the balanced electrodes.

Detailed Description Text (18):
A film coating may also be prepared by dip coating a substrate into the precursor hydrogel or by doctor blading a layer onto the substrate or by spray coating. Dip coating is described in Example 3.

Detailed Description Text (20):
The liquid gel was pasted onto the C-BORE of a stainless steel disk (2.54 cm in diameter). The C-BORE was at the center of the Disk with 1 cm in diameter and with the depth varying from 0.1 mm to 1 mm. The gel was dried in air for one hour and then vacuum dried for at least 16 hours before use as cathode for Li insertion and release tests. "C-BORE" is a term used in machine shops to describe a piece after a circular region is bored out of a bulk metal substrate.

Detailed Description Text (24):
Precursor hydrogel was coated onto stainless steel substrates and also onto gold coated silicon and gold coated quartz substrates. The hydrogel was freeze dried to a 10 to 100 micron thick film. As electrodes, the substrates were placed in a solution of 1M Li ClO.sub.4 in propylene carbonate and cycled versus Li. At least 3 moles Li per mole V.sub.2 O.sub.5 mole may be readily inserted.

Detailed Description Text (26):
Precursor hydrogel may be converted to an organogel by replacing the water with an organic solvent such as acetone, acetonitrile, or 1,4-dioxane, acetone being preferred. Many other solvents are possible by trial and error or through scientific selection of similar organic solvents, including desired subgroups. That solvent is then replaced with liquid CO.sub.2 in a supercritical drying chamber maintained at 800 psi and 10.degree. C. The pressure and temperature are increased beyond the critical point for CO.sub.2 to provide it in its gaseous form and it is then removed while maintaining the conditions above the critical temperature. High surface area materials with 300-450 m.sup.2 /g are typically obtained.

Detailed Description Text (27):
An aerogel powder is obtained by supercritically drying the bulk hydrogel. The nominal compositions of this form of the invention is presented V.sub.2 O4.5(OH) (bound carbon) where the chemically bound carbon is left over from the drying procedure. It is believed to be a remnant of the solvent used. The compositions contain about 4.5 wt. % water and 4.0-7 wt. % bound carbon.

Detailed Description Text (28):

Thin film aerogel is obtained by supercritically drying a dip coated layer of the liquid gel on a stainless steel substrate. Thick film aerogel electrodes are obtained by supercritically drying the liquid gel in a supporting structure of stainless steel metal screen or of graphite felt (3 mm thick by 1 cm diameter, 80% porosity). Many other materials and structures can be used including most of the types of current collectors used in lithium (ion) batteries.

Detailed Description Text (30):
Various forms of aerogel electrodes were placed in a solution of 1M Li ClO.sub.4 in propylene carbonate and cycled versus Li. Up to 4 mole of Li per mole of V.sub.2 O.sub.5 were reversibly inserted into the electrodes between 4.1 and 1.5V as can be seen in the graph of FIG. 3.

Detailed Description Text (32):
Precursor hydrogel may be mixed with carbon particles and sonicated to yield a composite hydrogel. The product may be quick vacuum processed, spin coated, dip coated, etc., (XRG), spray dried, freeze dried as a composite (CRG) or converted to an aerogel (ARG) composite powder. Final drying of such composites is preferably under vacuum.

Detailed Description Text (35):
A xerogel-carbon composite dip coated film was so formed and placed in a solution of 1M Li ClO.sub.4 in propylene carbonate and cycled versus Li. Up to 4 moles of Li per mole of V.sub.2 O.sub.5 were reversibly inserted into the electrode between 3.8 and 1.5 V as is seen in the graph of FIG. 4. The insertion rate was orders of magnitude higher than the equivalent carbon-free film, with higher specific energy.

Detailed Description Text (37):
One embodiment uses the precursor hydrogel, adds electronically conductive high surface area powder such as Shawinigan an acetylene black and a binder such as PTFE. This composite gel is then sheared by sonication and then coated onto an electrode foil substrate by dip coating, doctor blading or spray coating, treated as per the XRG, CRG or ARG described above to yield a single or double side coated electrode and then calendared. Such a foil may be continuously produced and readily assembled into standard prismatic or spiral wrapped batteries.

Detailed Description Text (38):
Bipolar electrodes may also be readily constructed for use in high voltage, series connected battery structures.

Detailed Description Text (40):
Alternatively, the composite gel may be formed as in Example 8 without binder. This may then be converted into the XRG, CRG or ARG powder form according to the invention. The powder may then be formed into an electrode coating or pressed body with addition of appropriate binders such as PVDF or PTFE by use of appropriate solvent for layer adhesion to a substrate.

Detailed Description Text (41):
The resultant article may be calendared and used as described in Example 8. Such an electrode is demonstrated to be reversible in FIG. 5.

Detailed Description Text (45):
Another approach is to react V.sub.2 O.sub.5 precursor hydrogel with a thin film of metal, such as silver or nickel and treated to form the M.sub.x V.sub.2 O.sub.4.5 (OH) of the invention (where M=Ag, Ni, etc.).

Detailed Description Text (47):
The electrochemical performance of ARG film and XRG film samples on stainless steel substrates was studied in three electrode cells using metallic lithium as both the

reference and counter electrodes. A 1 M solution of Li ClO.sub.4 in anhydrous propylene carbonate was used as electrolyte. Coulometric titration by the galvanostatic intermittent titration technique (GITT) as described by W. Weppner and R. A. Huggins., J. Electrochem. Soc., 124, 1569 (1977), was used to characterize the equilibrium potential as a function of the extent of lithium insertion. FIG. 6 shows the equilibrium potential versus the number of lithium ions inserted for both materials, yielding the highest specific capacities of any known vanadium oxide. In addition, the insertion is completely reversible and all the inserted lithium may be removed from the hosts.

Detailed Description Text (53):
The preparation of the aerogel form is realized by exchanging water in the hydrogel with an organic solvent to make an organogel which is then supercritically dried with CO.sub.2. This provides a host product having a surface area of up to 300-450 square meters/gram and reduces the diffusion distance in the host. Both greatly enhance the rate of injection and release of Li.sup.+.

Detailed Description Text (54):
Films may be produced ranging from 20 or 30 nanometers up to tens of microns thick or particles that are submicron or above or highly porous films and particles and have surface areas up to 300-450 square meters/gram. Shearing of the precursor hydrogel may typically be accomplished by spinning, agitation, shear coating, doctor blading, roll coating or by spraying. Other methods will ready occur to those familiar with this art.

Detailed Description Text (55):
The more preferred processing involves quick vacuum drying at room temperature (xerogels), freeze drying (cryogels) and supercritical drying (aerogels). The drying conditions influence the final internal structure of the materials causing the self-assembled polymers of the hydrogel to organize preferentially with respect to each other. Highly ordered products have lower capacity for Li insertion and lower specific energy than disordered materials.

Detailed Description Text (63):
There are provided herein unique compositions for high rate materials for electrodes for batteries and unique methods of synthesizing such materials.

Detailed Description Text (64):
The invention described is not intended to be limited to embodiments disclosed but includes modifications made within the true spirit and scope of the invention. The insertion will be of specific use in batteries such as "lithium-ion" or "rocking-chair" batteries and also batteries with Li-ion conducting polymer electrolytes. All of which are included herein, although not exclusively, when the term "battery" is used.

Other Reference Publication (1):
Columetric titration by the galvanostatic intermittent titration technique (GITT) as described by W. Weppner and R.A. Huggins in Determination of the Kinetic Parameters of Mixed-Conducting Electrodes and Application to the System Li.sub.3,Sb, J. Electrochem. Soc., 124, 1569 (1977). (Month Unknown).

Other Reference Publication (4):
High Surface Area V.sub.2 O.sub.5 Aerogel Intercalation Electrodes, by D.B. Le, S. Passerini, X. Chu, D. Chang, B.B. Owens, and W.H. Smyrl, Department of Chemical Engineering and Materials Science, Corrosion Research Center, University of Minnesota, Minneapolis, MN 55455. 1995 (Month Unknown).

CLAIMS:

1. A process for the preparation of intercalation materials of the nominal compound

V.sub.2 O.sub.4.5 (OH), comprising:

providing an aqueous solution of V.sub.2 O.sub.5 networks progressively formed as a sol, then a gel to yield a precursor hydrogel and treating the precursor hydrogel to form a xerogel of the nominal compound V.sub.2 O.sub.4.5 (OH) by selecting one of the methods from the group consisting of:

A. vacuum drying of the precursor to the xerogel V.sub.2 O.sub.4.5 (OH) form to a bulk powder; and

B. preparing a film xerogel on a substrate utilizing shear.

3. A process for the preparation of intercalation materials of the nominal compound V.sub.2 O.sub.4.5 (OH), comprising:

providing an aqueous solution of V.sub.2 O.sub.5 networks progressively formed as a sol, then a gel to yield a precursor hydrogel and treating the precursor hydrogel to form a xerogel of the nominal compound V.sub.2 O.sub.4.5 (OH) by selecting one of the methods from the group consisting of:

A. vacuum drying of the precursor to the xerogel V.sub.2 O.sub.4.5 (OH) form to a bulk powder; and

B. preparing a film xerogel on a substrate utilizing shear by means of dip coating, followed by a vacuum drying step.

4. A process for the preparation of intercalation materials of the nominal compound V.sub.2 O.sub.4.5 (OH), comprising:

providing an aqueous solution of V.sub.2 O.sub.5 networks progressively formed as a sol, then a gel to yield a precursor hydrogel and treating the precursor hydrogel to form a xerogel of the nominal compound V.sub.2 O.sub.4.5 (OH) by selecting one of the methods from the group consisting of:

A. vacuum drying of the precursor to the xerogel V.sub.2 O.sub.4.5 (OH) form to a bulk powder; and

B. preparing a film xerogel on a substrate utilizing shear by spray coating, followed by a vacuum drying step.

5. A process for the preparation of intercalation materials of the nominal compound V.sub.2 O.sub.4.5 (OH), comprising:

providing an aqueous solution of V.sub.2 O.sub.5 networks progressively formed as a sol, then a gel to yield a precursor hydrogel and treating the precursor hydrogel to form a xerogel of the nominal compound V.sub.2 O.sub.4.5 (OH) by selecting one of the methods from the group consisting of:

A. vacuum drying of the precursor to the xerogel V.sub.2 O.sub.4.5 (OH) form to a bulk powder; and

B. preparing a film xerogel on a substrate utilizing shear by doctor blading, followed by a vacuum drying step.

6. A process for the preparation of intercalation materials of the nominal compound V.sub.2 O.sub.4.5 (OH), comprising:

providing an aqueous solution of V.sub.2 O.sub.5 networks progressively formed as a sol, then a gel to yield a precursor hydrogel and treating the precursor hydrogel to form a xerogel of the nominal compound V.sub.2 O.sub.4.5 (OH) by selecting one

of the methods from the group consisting of:

A. vacuum drying of the precursor to the xerogel V.sub.2 O.sub.4.5 (OH) form to a bulk powder; and

B. preparing a film xerogel on a substrate utilizing shear; wherein the precursor hydrogel is treated by adding conductive particles to it prior to xerogel formation.

9. The process of claim 8 wherein the sonicated precursor is formed into a coating on a substrate.

13. An electrode including a quantity of nominal compound V.sub.2 O.sub.4.5 (OH) xerogel characterized in that it is capable of intercalating lithium ions to the extent greater than 2.4 and up to 4 Li.sup.+ ions per unit of V.sub.2 O.sub.5.

14. The electrode of claim 13 wherein the xerogel is in the form of a coating.

15. The electrode of claim 13 wherein the xerogel is in the form of a powder mixed with other constituents.

16. The electrode of claim 15 wherein one of the other constituents is a quantity of conductive particles.

17. The electrode of claim 16 wherein the particles are a form of carbon.

18. The electrode of claim 15 wherein one of the other constituents is a binder.

19. The electrode of claim 13 further comprising a metal bronze.